Anti-ulcer and Analgesic activity of Ethanolic extract of *Annona squamosa* Leaves

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THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

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In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

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Submitted by

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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"Anti-ulcer and Analgesic activity of Ethanolic extract of *Annona squamosa* leaves"** submitted by the student bearing [**Reg.No. 26103093]** to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment for the award of degree of **MASTER OF PHARMACY** in **PHARMACOLOGY** was evaluated by us during the examination held on…….

Internal Examiner **External Examiner**

CERTIFICATE

This is to certify that the work embodied in this dissertation "**Anti-ulcer and Analgesic activity of Ethanolic extract of *Annona squamosa* leaves",** submitted to "The Tamil Nadu Dr.M.G.R.Medical University", Chennai, was carried out by **Mr.BillaVamsi [Reg.No. 26103093],** in the Partial fulfillment of award of degree of **MASTER OF PHARMACY** in Pharmacology under direct supervision of **Dr. P. ASHOKKUMAR, M. Pharm, Ph. D.,** Professor**,** Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Komarapalayam, during the academic year 2011-2012.

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The work presented in this dissertation entitled **"Anti-ulcer and Analgesic activity of Ethanolic extract of *Annona squamosa* leaves",** was carried out by me, under the direct supervision of **Dr. P. ASHOKKUMAR, M. Pharm, Ph. D.,** Professor, Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Komarapalayam.

I further declare that, this work is original and has not been submitted in part or full for the award of any other degree or diploma in any other university.

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DEDICATED TO

ALMIGHTY

MY BELOVED PARENTS,

MY GUIDE

&

FRIENDS

CONTENTS

LIST OF ABBREVIATIONS

µg - Microgram

ANOVA - Analysis of variance

Cl^- - Chloride ions

COX-1 - Cyclooxygenase-1

COX-2 - Cyclooxygenase-2

ECL - Enterochromaffin cells

g - Gram

HCl - Hydrochloric acid

HCO_3^- - Carbonic acid

hr - Hour

i.p. - Intraperitonially

K^+ - Potassium ions

kg - Kilogram

M - Molar

A.Squamosa - *Annona squamosa*

mg - Milligram

min - Minute

ml - Milli litre

NSAID - Non steroidal anti-inflammatory drugs

$O_2^{\cdot}$ - Superoxide

$OH^{\cdot}$ - Hydroxyl

PAF - Platelet activating factor

PG - Prostaglandin

PL - Pylorus ligation

PUD - Peptic ulcer disease

SEM - Standard error mean

UI - Ulcer index

1. INTRODUCTION

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savoury qualities. Botanists define an herb as being a soft stemmed plant, which dies after flowering, while **"herbalists define an herb as any part of a plant which can be used for medicine"**, cooking, and cosmetic uses and as a scent or dye. Herb plants produce and contain a variety of chemical substances that act upon the body.

The leaves, flowers, stems, berries, and roots of plants are used to prevent, relieve, and treat illness. From a "scientific" perspective, many herbal treatments are considered experimental. The reality is, however, that herbal medicine has a long and respected history. Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations. For example, vincristine (an antitumor), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants.**(Kokate C.K., 1995)**

IMPORTANCE OF HERBAL

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics drug that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security.

Our ancestors used trial and error to discover the most effective local plants for the treatment of illnesses. Advances in science have enabled a better understanding of the physiological effects of herbs on the human body and therefore

their role in restoring health. Herbal medicines support the body's natural healing process, and aim to treat the person as well as the disease. This means it can bring about a deep and lasting change.

PROSPECTS OF HERBAL RESEARCH

There is a worldwide 'green revolution', (Mukherjee, P.K., 2002) which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Furthermore, underlying this upsurge of interest in plants is the fact that many important drugs in use today were derived from plants or from starting molecules of plant origin.

Table No. 1. List of Anti-ulcer plants

Plant	Family	Plant part used
Alpiniaallughas	Zingiberaceae	Rhizome
Alpinia galangal	Zingiberaceae	Rhizome
Alpiniacalcarata	Zingiberaceae	Rhizome
Glycyrrhizaglabra	Liquorice	Stem, root
Azadirachtaindica	Meliaceae	Bark, leaves, flower
Acacia catechu	Mimosaceae	Bark
Sophorasubprostrata	Leguminaceae	Root
Magnolia bark	Magnoliaceae	Bark
Gloriosa superb	Liliaceae	Roots, rhizome
Phyllanthusemblica	Euphorbiaceae	Root, bark
Aeglemarmelos	Rutaceae	Leaf
Indigoferatinctoria	Papillonaceae	Leaf, fruit

Pongamiapinnata	Papillonaceae	Whole plant
Ecliptaprostata	Asteraceae	Root
Terminaliaarjuna	Combretaceae	Bark
Terminaliaalata	Combretaceae	Bark
Terminaliachebula	Combretaceae	Powdered fruit
Coleus vettiveroides	Lamiaceae	Whole plant
Puniagranatum	Puniaceae	Fruit
Murrayakoenigii	Rutaceae	Leaf
Solanummelongina	Solanaceae	Fruit
Solanumnigram	Solanaceae	Plant
Bauhinia variegate	Caesalpiniaceae	Bark
Bauhinia purpurea	Caesalpiniaceae	Bark
Gymnemasylvestre	Asclepiadaceae	Whole plant
Balospermummontanum	Euphorbiaceae	Root
Saracaasoca	Caesalpiniaceae	Bark
Andrographispaniculata	Acanthaceae	Whole plant
Aristolochiabracteolata	Aristolochiaceae	Whole plant
Ficusmicrocarpa	Moraceae	Bark, leaf
Mimusopselengi	Sapotaceae	Flower
Lagenaria vulgaris	Curcubitaceae	Fruit
Mormodicacharatia	Curcubitaceae	Fruit, seed
Citrulluscolocynthis	Curcubitaceae	Fruit, root

Since modern drugs are not able to completely cure chronic diseases but, rather, to prevent further deterioration associated with them, patients must take drugs for extended periods of time. Due to the ineffectiveness as well as the potential side effects, patients are often led to explore complementary/ alternative medicines such as herb, and medicinal botanicals in particular. However, simultaneous administration of herbs and drugs may mimic, magnify or oppose the pharmacological effects of each other. It is widely believed that although herbs hold promise as therapeutically effective medicaments, in-depth and appropriate studies should be carried out to confirm their efficacy in the presence of modern medicines.

PHYSIOLOGY OF STOMACH

The stomach is a muscular, hollow, dilated part of the alimentary canal which functions as an important organ of the digestive tract. It is involved in the second phase of digestion, following mastication (chewing). The stomach is located between the oesophagus and the small intestine. The stomach is the most dilated part of the digestive tube, and is situated between the end of the oesophagus and the beginning of the small intestine. It lies in the epigastric, umbilical, and left hypochondriac regions of the abdomen, and occupies a recess bounded by the upper abdominal viscera, and completed in front and on the left side by the anterior abdominal wall and the diaphragm. It secretes protein-digesting enzymes and strong acids to aid in food digestion, (sent to it via oesophagealperistalsis) through smooth muscular contortions (called segmentation) before sending partially digested food (chyme) to the small intestines.

The word *stomach* is derived from the Latin*stomachus* which is derived from the Greek word *stomachos*, ultimately from *stoma*, "mouth". The words *gastro-* and *gastric* (meaning related to the stomach) are both derived from the Greek word *gaster*. The stomach is an organ between the oesophagus and the small intestine. It is where digestion of protein begins. The stomach has three tasks. It stores swallowed food. It mixes the food with stomach acids. Then it sends the mixture on to the small intestine.

The shape and position of the stomach are so greatly modified by changes within itself and in the surrounding viscera that no one form can be described as typical. The chief modifications are determined by (1) the amount of the stomach contents, (2) the stage which the digestive process has reached, (3) the degree of development of the gastric musculature, and (4) the condition of the adjacent intestines. It is, however, possible by comparing a series of stomachs to determine certain markings more or less common to all.

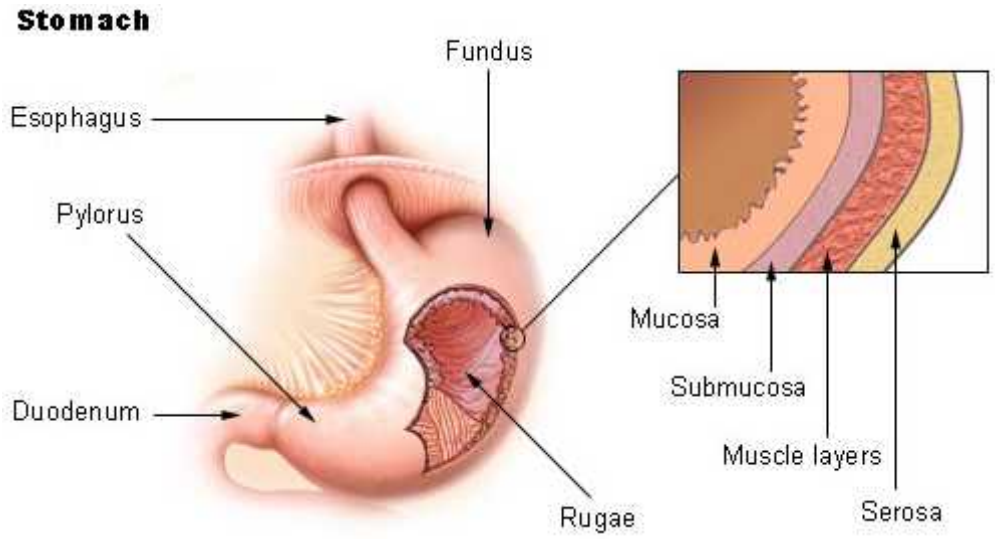


Figure: 1

Component Parts of the Stomach

A plane passing through the incisuraangularis on the lesser curvature and the left limit of the opposed dilatation on the greater curvature divides the stomach into a left portion or body and a right or pyloric portion. The left portion of the body is known as the fundus, and is marked off from the remainder of the body by a plane passing horizontally through the cardiac orifice. The pyloric portion is divided by a plane through the sulcus intermedius at right angles to the long axis of this portion; the part to the right of this plane is the pyloric antrum.

The stomach presents two openings, two borders (or curvatures) and two surfaces.

Openings

The opening by which the esophagus communicates with the stomach is known as the **cardiac orifice,** and is situated on the left of the middle line at the level of the tenth thoracic vertebra. The short abdominal portion of the esophagus (*antrumcardiacum*) is conical in shape and curved sharply to the left, the base of the cone being continuous with the cardiac orifice of the stomach. The right margin of the esophagus is continuous with the lesser curvature of the stomach, while the left margin joins the greater curvature at an acute angle, termed the incisuracardiaca.

The **pyloric orifice** communicates with the duodenum, and its position is usually indicated on the surface of the stomach by a circular groove, the duodenopyloric constriction**.** This orifice lies to the right of the middle line at the level of the upper border of the first lumbar vertebra.

Curvatures

The **lesser curvature** (*curvaturaventriculi minor*), extending between the cardiac and pyloric orifices, forms the right or posterior border of the stomach. It descends as a continuation of the right margin of the esophagus in front of the fibers of the right crus of the diaphragm, and then, turning to the right, it crosses the first lumbar vertebra and ends at the pylorus. Nearer its pyloric than its cardiac end is a well-marked notch, the incisuraangularis, which varies somewhat in position with the state of distension of the viscus; it serves to separate the stomach into a right and a left portion. The lesser curvature gives attachment to the two layers of the hepatogastric ligament, and between these two layers are the left gastric artery and the right gastric branch of the hepatic artery.

The **greater curvature** (*curvaturaventriculi major*) is directed mainly forward, and is four or five times as long as the lesser curvature. Starting from the cardiac orifice at the incisuracardiaca, it forms an arch backward, upward, and to the left; the highest point of the convexity is on a level with the sixth left costal cartilage. From this level it may be followed downward and forward, with a slight convexity to the left as low as the cartilage of the ninth rib, it then turns to the right, to the end of

the pylorus. Directly opposite the incisuraangularis of the lesser curvature the greater curvature presents a dilatation, which is the left extremity of the pyloric part**;** this dilatation is limited on the right by a slight groove, the sulcus intermedius, which is about 2.5cm, from the duodenopyloric constriction. The portion between the sulcus intermedius and the duodenopyloric constriction is termed the pyloric antrum**.** At its commencement the greater curvature is covered by peritoneum continuous with that covering the front of the organ. The left part of the curvature gives attachment to the gastrolienal ligament, while to its anterior portion are attached the two layers of the greater omentum, separated from each other by the gastroepiploic vessels.

Surfaces

When the stomach is in the contracted condition, its surfaces are directed upward and downward respectively, but when the viscus is distended they are directed forward, and backward. They may therefore be described as anterosuperior and postero-inferior.

Antero-superior Surface: The left half of this surface is in contact with the diaphragm, which separates it from the base of the left lung, the pericardium, and the seventh, eighth, and ninth ribs, and intercostal spaces of the left side. The right half is in relation with the left and quadrate lobes of the liver and with the anterior abdominal wall. When the stomach is empty, the transverse colon may lie on the front part of this surface. The whole surface is covered by peritoneum.

Postero-inferior Surface: It is in relation with the diaphragm, the spleen, the left suprarenal gland, the upper part of the front of the left kidney, the anterior surface of the pancreas, the left colic flexure, and the upper layer of the transverse mesocolon. These structures form a shallow bed, the stomach bed, on which the viscus rests. The transverse mesocolon separates the stomach from the duodenojejunal flexure and small intestine. The postero-inferior surface is covered by peritoneum, except over a small area close to the cardiac orifice; this area is limited by the lines of attachment of the gastrophrenic ligament, and lies in apposition with the diaphragm, and frequently with the upper portion of the left suprarenal gland.

Role in Digestion

Bolus (masticated food) enters the stomach through the oesophagus via the oesophageal sphincter. The stomach releases proteases (protein-digesting enzymes such as pepsin) and hydrochloric acid, which kills or inhibits bacteria and provides the acidic pH of 2 for the proteases to work. Food is churned by the stomach through muscular contractions of the wall - reducing the volume of the fundus, before looping around the fundus and the body of stomach as the boluses is converted into chyme (partially digested food). Chyme slowly passes through the pyloric sphincter and into the duodenum, where the extraction of nutrients begins. Depending on the quantity and contents of the meal, the stomach will digest the food into chyme anywhere between 40 minutes and a few hours.

Acid Secretion

Parietal cells in the stomach secrete roughly two liters of acid a day in the form of hydrochloric acid. When stimulated, these parietal cells secrete HCl at a concentration of roughly 160 mM (equivalent to a pH of 0.8). Acid in the stomach functions to kill bacteria, and to aid digestion by solubilizing food. The acid is also important to establish the optimal pH (1.8-3.5) for the function of the digestive enzyme **pepsin**.

The acid is secreted into large cannaliculi, deep invaginations of the plasma membrane which are continuous with the lumen of the stomach. When acid secretion is stimulated there is a dramatic change in the morphology of the membranes of the parietal cell. Cytoplasmic tubulovesicular membranes which are abundant in the resting cell virtually disappear in concert with a large increase in the cannalicular membrane

The epithelium of the stomach is intrinsically resistant to the damaging effects of gastric acid and other consequences. Nonetheless, excessive secretion of gastric acid is a major problem in humans leading to gastritis, gastric ulcers and peptic acid disease.

Mechanism

The ability of the parietal cell to secrete acid is dependent on active transport. The key player in acid secretion is H+/K+ ATPase or "proton pump" located in the cannalicular membrane. This ATPase is magnesium-dependent, and not inhibited by ouabain. The current model for explaining acid secretion is as follows

- Hydrogen ions are generated within the parietal cell from dissociation of water. The hydroxyl ions formed in this process rapidly combine with carbon dioxide to form bicarbonate ion, a reaction catalyzed by carbonic anhydrase.
- Bicarbonate is transported out of the basolateral membrane in exchange for chloride. The outflow of bicarbonate into blood results in a slight elevation of blood pH known as the "alkaline tide". This process serves to maintain intracellular pH in the parietal cell.
- Chloride and potassium ions are transported into the lumen of the cannaliculus by conductance channels, and such is necessary for secretion of acid.
- Hydrogen ion is pumped out of the cell, into the lumen, in exchange for potassium through the action of the proton pump; potassium is thus effectively recycled.
- Accumulation of osmotically-active hydrogen ion in the cannaliculus generates an osmotic gradient across the membrane that results in outward diffusion of water - the resulting gastric juice is 155 mM HCl and 15 mM HCl with a small amount of NaCl.

Regulation of Acid secretion

Parietal cells bear receptors for three stimulators (positive regulators) of acid secretion, reflecting a triumverate of neural, endocrine and paracrine control:

I. Acetylcholine (muscarinic type receptor)
II. Gastrin
III. Histamine (H_2 type receptor)

Acetylcholine is a neurotransmitter that is released by **enteric neurons**. Gastrin is a hormone that is released by **G cells**, endocrine cells that are located in the gastric epithelium. Histamine is a paracrine that is released from **ECL (enterochromaffin-like)** cells.

Enterochromaffin-like or ECL cells are a distinctive type of neuroendocrine cell in the gastric mucosa underlying the epithelium. They are most prevalent in the acid-secreting regions of the stomach. ECL cells synthesize and secrete histamine in response to stimulation by the hormones gastrin and pituitary adenylyl cyclase-activating peptide. Gastrin itself is secreted by cells in the epithelium of the stomach, but travels to ECL cells via the blood. Together, histamine and gastrin are primary positive regulators of acid secretion from the parietal cell. ECL cells also secrete pancreastatin and probably are the source of one or more other peptide hormones and growth factors. ECL cells are readily identified in histologic sections stained by silver impregnation.

There is one more regulatory molecule, **Somatostatin**, which acts for negative acid secretion (negative regulator). Somatostatin is also secreted by endocrine cells of the gastric epithelium; it can act as either a paracrine or a hormone.

The adjacent figure depicts how the positive and negative regulators interact to stimulate acid secretion. Acetylcholine and histamine directly stimulate parietal cells to increase acid secretion. Gastrin stimulates acid secretion by stimulating histamine release from

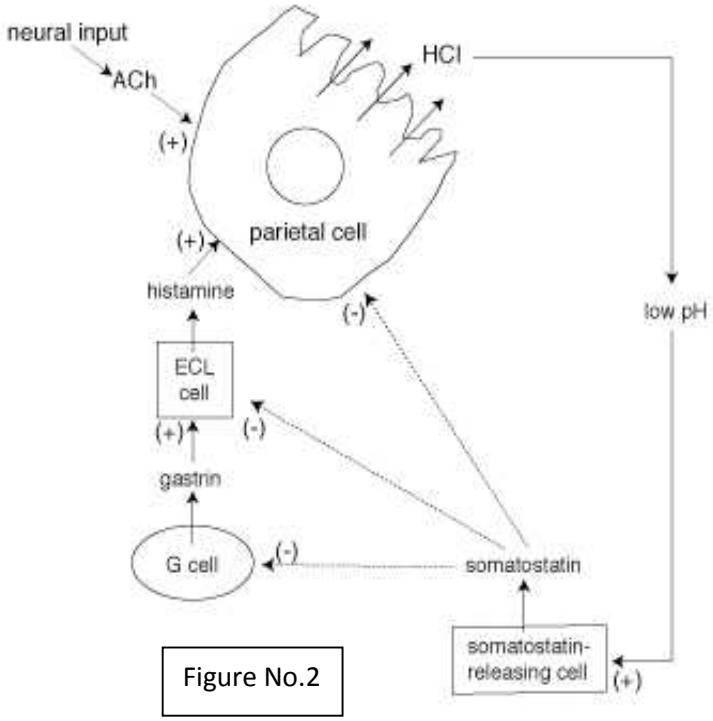


Figure No.2

ECL cells. (Gastrin also has a direct effect on parietal cells, which is to stimulate their proliferation). When the pH of the stomach gets too low, that stimulates somatostatin secretion. Somatostatin inhibits acid secretion by direct effects on parietal cells, and also by inhibiting release of the positive regulators histamine and gastrin. The balance of activity of the different regulators changes as food is consumed and passes through different segments of the upper GI tract.**(http://courses.washington.edu)**

PEPTIC ULCER DISEASE

A peptic ulcer is a sore or hole in the lining of the stomach or duodenum and is a term used to describe a lesion in the oesophagus, stomach, or duodenum. More specific names are used to describe an ulcer located at a specific site. Duodenal ulcers (the first part of the small intestine) are more common than other types of peptic ulcers. Peptic ulcers are caused by hyper secretion of hydrochloric acid and pepsin that erode the GI mucosal lining.

BASIC CAUSE OF PEPTIC ULCERATION

The usual cause of peptic ulceration is an *imbalance* between the rate of secretion of gastric juice and the degree of protection afforded by (1) the gastro duodenal mucosal barrier and (2) the neutralization of the gastric acid by duodenal juices. It will be recalled that all areas normally exposed to gastric juice are well supplied with mucous glands, beginning with compound mucous glands in the lower oesophagus plus the mucous cell coating of the stomach mucosa, the mucous neck cells of the gastric glands, the deep pyloric glands that secrete mainly mucus, and, finally, the glands of Brunner of the upper duodenum, which secrete a highly alkaline mucus. In addition to the mucus protection of the mucosa, the duodenum is protected by the *alkalinity of the small intestinal secretions*. Especially important is *pancreatic secretion*, which contains large quantities of sodium bicarbonate that neutralize the hydrochloric acid of the gastric juice, thus also inactivating pepsin and preventing digestion of the mucosa. In addition, large amounts of bicarbonate ions are provided in (1) the secretions of the large Brunner's glands in the first few centimetres of the duodenal wall and (2) in bile coming from the liver.

Causes

1. High acid and peptic content
2. Irritation
3. Poor blood supply
4. Poor secretion of mucus
5. Infection, *H. Pylori*

PATHOPHYSIOLOGY

A physiologic imbalance between aggressive factors (gastric acid and pepsin) and protective factors (mucosal defense and repair) remain important issues in the pathophysiology of gastric and duodenal ulcers. Gastric acid is secreted by the parietal cells, which contain receptors for histamine, gastrin, and acetylcholine. Acid (as well as *H. pylori* infection and NSAID use) is an independent factor that contributes to the disruption of mucosal integrity. Increased acid secretion has been observed in patients with duodenal ulcers and may be a consequence of *H. pylori* infection. Patients with ZES have profound gastric acid hyper secretion resulting from a gastrin producing tumour. Patients with gastric ulcer usually have normal or reduced rates of acid secretion (hypochlorhydria). Acid secretion is expressed as the amount of acid secreted under basal or fasting conditions, basal acid output (BAO); after maximal stimulation, maximal acid output (MAO); or in response to a meal. Basal, maximal, and meal-stimulated acid secretion varies according to time of day and the individual's psychological state, age, gender, and health status. The BAO follows a circadian rhythm, with the highest acid secretion occurring at night and the lowest in the morning. An increase in the BAO: MAO ratio suggests a basal hyper secretory state such as ZES. Pepsin is an important cofactor that plays a role in the proteolytic activity involved in ulcer formation. Pepsinogen, the inactive precursor of pepsin, is secreted by the chief cells located in the gastric fundus. Pepsin is activated by acid pH (optimal pH of 1.8 to 3.5), inactivated reversibly at pH 4, and irreversibly destroyed at pH 7. Mucosal defence and repair mechanisms (mucus and bicarbonate secretion, intrinsic epithelial cell defence, and mucosal blood flow) protect the gastro duodenal mucosa from noxious endogenous and exogenous substances. The viscous nature and near-neutral pH of the mucus-bicarbonate barrier protect the stomach from the acidic contents in the gastric lumen. Mucosal repair after injury is related to

epithelial cell restitution, growth, and regeneration. The maintenance of mucosal integrity and repair is mediated by the production of endogenous prostaglandins. The term *cytoprotection*is often used to describe this process, but *mucosal defense* and *mucosal protection* are more accurate terms, as prostaglandins prevent deep mucosal injury and not superficial damage to individual cells. Gastric hyperemia and increased prostaglandin synthesis characterize adaptive cytoprotection, the short-term adaptation of mucosal cells to mild topical irritants. This phenomenon enables the stomach to initially withstand the damaging effects of irritants. Alterations in mucosal defence that are induced by *H. pylori* or NSAIDs are the most important cofactors in the formation of peptic ulcers.

Chronic Gastritis

Chronic gastritis is defined as the presence of chronic mucosal inflammatory changes leading eventually to mucosal atrophy and epithelial metaplasia.It is notable for distinct causal subgroups and for patterns of histologic alterations that vary in different parts of the world. In the Western world, the prevalence of histologic changes indicative of chronic gastritis exceeds 50% in the later decades of adult life.

Acute Gastritis

Acute gastritis is an acute mucosal inflammatory process, usually of a transient nature. The inflammation may be accompanied by haemorrhage into the mucosa and, in more severe circumstances, by sloughing of the superficial mucosal epithelium *(erosion)*. This severe erosive form of the disease is an important cause of acute gastrointestinal bleeding.

Peptic ulcers are remitting, relapsing lesions that are most often diagnosed in middle-aged to older adults, but they may first become evident in young adult life. They often appear without obvious precipitating influences and may then heal after a period of weeks to months of active disease. *Even with healing, however, the propensity to develop peptic ulcers remains.* Thus, it is difficult to obtain accurate data on the prevalence of active disease. Best estimates suggest that in the American population, about 2.5% of males and 1.5% of females have peptic ulcers. For both men and women in the United States, the lifetime risk of developing peptic ulcer disease is about 10%.

EPIDEMIOLOGY

Genetic or racial influences appear to play little or no role in the causation of peptic ulcers. Duodenal ulcers are more frequent in patients with alcoholic cirrhosis, chronic obstructive pulmonary disease, chronic renal failure, and hyperparathyroidism.

THE REGULATION OF ACID SECRETION

The regulation of acid secretion by parietal cells is especially important in the pathogenesis of peptic ulcer, and constitutes a particular target for drug action. The secretion of the parietal cells is an isotonic solution of HCl (150 mmol /l) with a pH less than 1, the concentration of hydrogen ions being more than a million times higher than that of the plasma. The Cl^- is actively transported into *canaliculated* in the cells that communicate with the lumen of the gastric glands and thus with the stomach itself. This Cl^- secretion is accompanied by K^+, which is then exchanged for H^+ from within the cell by a K^+/H^+ ATPase and bicarbonate ions. The latter exchanges across the basal membrane of the parietal cell for Cl^-. The principal stimuli acting on the parietal cells are

- Gastrin (a stimulatory hormone)
- Acetylcholine (a stimulatory neurotransmitter)
- Histamine (a stimulatory local hormone)
- Prostaglandins E_2 and I_2 (local hormones that inhibit acid secretion).

Gastrin is a peptide hormone synthesised in the mucosa of the gastric antrum and duodenum, and secreted into portal blood. Its main action is stimulation of the secretion of acid by the parietal cells. These receptors are blocked by *proglumide* which inhibits gastrin action.

Acetylcholine is released from neurons and stimulates specific muscarinic receptors on the surface of the parietal cells and on the surface of histamine-containing cells.

Histamine: Within the stomach, mast cells (or histamine-containing cells similar to mast cells) lying close to the parietal cell release a steady basal release of histamine, which is further increased by gastrin and acetylcholine. The hormone acts

on parietal cell H_2 receptors, which are responsive to histamine concentrations that are below the threshold required for vascular H_2 receptor activation.

The parietal cell itself has H_2 receptors for histamine and muscarinic M_2 receptors for acetylcholine, as well as receptors for gastrin itself. Acid secretion follows after the synergistic stimulation of H_2 receptors (which increases cAMP), and M_2 and gastrin receptors (which increase cytosolic Ca^{2+}).

Prostaglandins (mainly E_2 and I_2), synthesised in the gastric mucosa mainly by cyclo-oxygenase-1, stimulate mucus and bicarbonate secretion, decrease acid secretion and cause vasodilatation, all of which serve to protect the stomach against damage. This probably explains the ability of many non-specific non-steroidal anti-inflammatory drugs to cause gastric bleeding and erosions. More selective cyclo-oxygenase-2 inhibitors such as celecoxib and rofecoxib appear to cause less stomach damage.

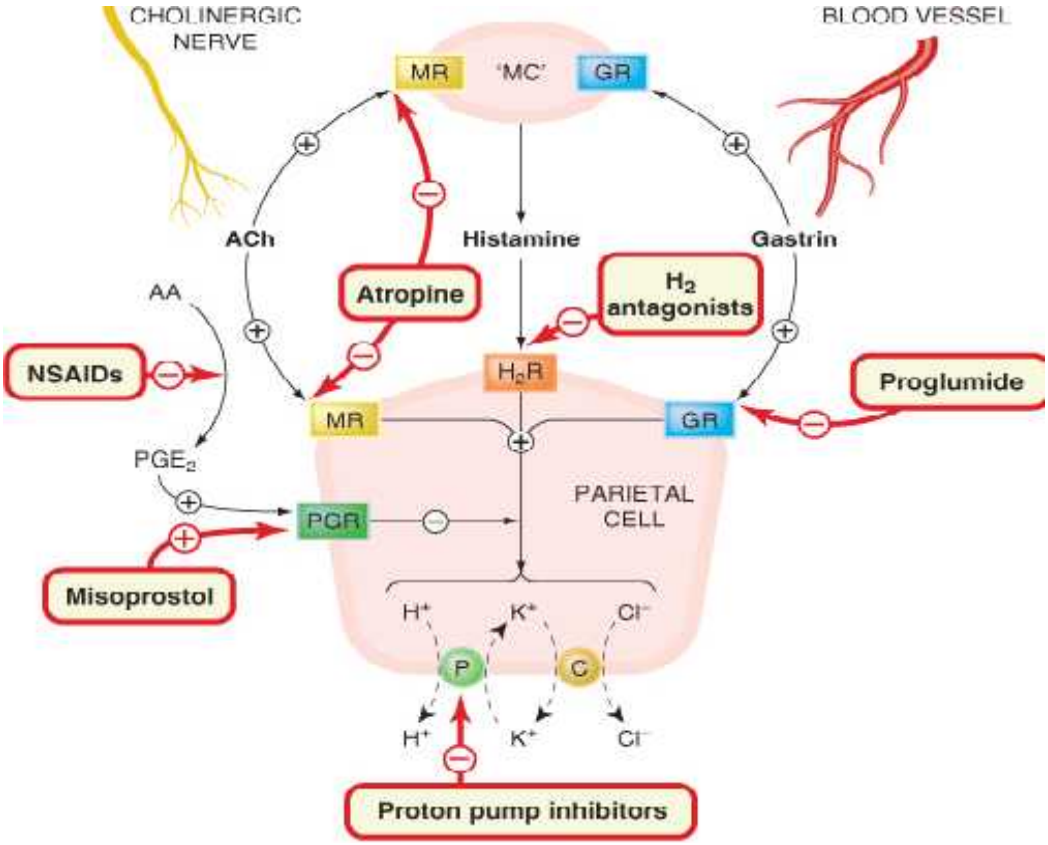


Figure: 3

DRUGS USED FOR PEPTIC ULCER DISEASE

Reduction of gastric acid secretion

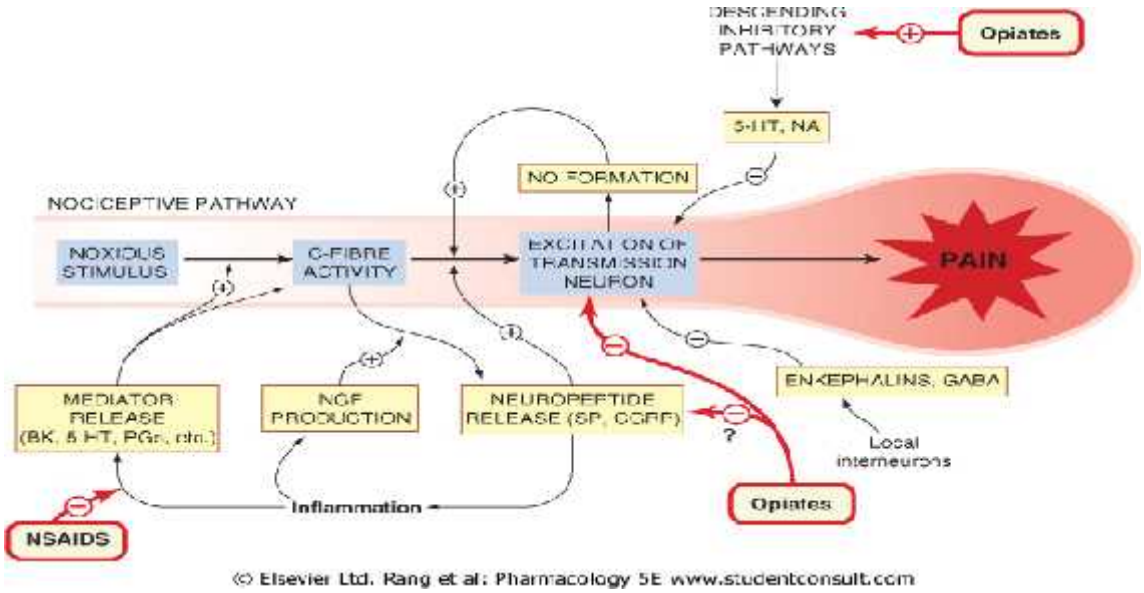
- **Proton pump inhibitors:** Pantoprazole, Omeprazole, lansoprazole, Rabeprazole.
- **H2-Receptor antagonists:** Cimetidine, Ranitidine, Famotidine and Nizatidine.
- **Anticholinergics:** Pirenzepin, Propantheline and Oxyphenonium.
- **Prostaglandin Analogs:** Misoprostol
- **Antacids:** Sodium bicarbonate, Sodium citrate, Magnesium hydroxide and Calcium carbonate.
- **Ulcer protective:** Sucralfate and Colloidal bismuth subcitrate.
- **Ulcer healing drugs:** Carbenoxolone sodium
- **Anti-H.pyloridrugs:** Amoxicillin, Clarithromycin, Metronidazole, Tetracycline.

ANALGESIC INTRODUCTION

Pain is the body's signal that something is wrong. Pain can result from an injury, such as a broken bone, a burn or a sprain from overuse of muscles (including muscle tension due to stress) from infections, such as sinus infections or meningitis or from natural events such as childbirth.

Pain begins at the level of the cells. In response to injury or inflammation, cells release chemical messengers. These chemical messengers alert other specialized cells called pain receptors. The pain receptors send signals to the brain. The brain interprets the signals and we perceive pain. Analgesics work by either blocking the signals that go to the brain or by interfering with the brain's interpretation of the signals. Among the most common analgesics are aspirin, choline salicylate, magnesium salicylate, and sodium salicylate. Ibuprofen, naproxen sodium and ketoprofen are all in the general category known as non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs relieve pain and also reduce inflammation. Another

common analgesic, acetaminophen, provides pain relief but does not reduce inflammation.



ammation.

Figure: 4

Table: 2

Analgesic classification

Drug Class	**Characteristics**	**Examples**	**Comments**
Opioids*	Acts on pain receptors in both spinal cord and brain; May be used with tranquilizers to induce a state of potent sedation (neuroleptanalgesia)	Morphine	Stimulates vomiting and vagal CNS centers; Can cause excitement in cats, horses and food animals; 4-6 hours duration;

			May be given SC or IM to help prevent hypotension; For moderate to severe pain
		Oxymorphone	Greater analgesic and sedative effects than morphine, does not cause hypotension, may cause excitement in cats, may be given IV, SC, IM or epidurally, approximate 4 hour duration for moderate to severe pain
		Meperidine (Demerol)	Rapid IV injection may cause severe hypotension, excitement and seizures,

			painful if given IM, preferred administration is SC, used in dogs, cats, and rodents, a synthetic opioid, not as potent as above drugs, often used with NSAIDs or as a preanesthetic, for mild to moderate pain
		Fentanyl	One of the most potent analgesics; Rapid onset-short duration (IV injection = 30 minutes); Usually administered by continuous IV drip or transdermal

			patch; Can cause panting and increased sensitivity to sound; For severe to moderate pain
		Butorphanol (Torbutrol)	Synthetic opioid- has both agonist and antagonist properties; Effective and safe post-op analgesic for mild to moderate pain, especially cranial visceral; May be used to help reverse effects of other opioids (respiratory depression and

			sedation), while still maintaining some analgesia; Do not give epidurally– potentially toxic to the spinal cord
		Buprenorphine	A partial agonist; Has a bell shaped response curve (may be less effective at higher doses than at moderate doses); Delayed onset, but gives 6-12 hours analgesia(IM) and 18-24 hours when given epidurally; Not adequate for severe

			pain (i.e. orthopedic pain), but is useful for mild to moderate pain; Commonly used for rodents and other species in research; Has been known to cause pica in rats
Alpha–2 agonists Thiazine derivatives	Profound sedative effect; Rapid onset; Reliable, reversible; Good muscle relaxation; May cause cardiovascular side effects; Use only in healthy animal; Use caution when handling-may be absorbed through skin abrasions;	Xylazine	May be used alone or in combination with other agents (i.e. Ketamine, opioids) IM or IV; Will allow greatly reduced doses of other agents including inhalants; May cause vomiting and

			bloat (in ruminants); Can be reversed with Yohimbine (IV)
		Meditomidine (Domitor)	Commonly used in combination with other agents (dose of general anesthetic should be reduced); Less likely to cause vomiting; May be reversed with Atipamesole (Antesedan) which will not

			reverse the effects of other drugs given concurrently with Meditomidi-ne; *High doses of Atipamesole can cause panting, excitement, muscle tremors, hypotension, and tachycardia-especially if given IV
NSAID (Non-steroidal anti-inflammatory drugs)	Analgesic, antipyretic, and anti-inflammatory; Effective for musculoskeletal pain; Requires 30-60 minutes for full analgesic properties to take effect; Metabolized in the liver; Negligible	Aspirin	May cause gastric irritation and prolonged bleeding time; Prolonged half life in cats, geriatrics and neonates

	effect on cardiovascular and respiratory systems		
		Acetaminophen	Toxic to cats and hepatotoxic to dogs; Less gastric irritation than Aspirin
		Ibuprofen	Renal, gastric effects; Narrow safety margin in cats
		Flunixin (Banamine)	Significant renal toxicity is possible in hypotensive

			patients; Do not use with Methoxyflur-ane
		Ketoprofen	Potent analgesic, especially for orthopedic patients; Gastric irritation and ulceration may occur at therapeutic doses
		Carprofen (Rimadyl)	Less potential for gastric ulceration than some NSAID's; Renal toxicity seen in dogs with prolonged use (especially Labrador Retrievers)
		Meloxicam	Can cause vomiting and

			diarrhea; Less potential for gastric ulceration than some NSAID's; 5-day limit for treatment of cats
Local analgesia	Can be sprayed, injected at surgical site, or infiltrated around a nerve supplying the affected area; May be used to desensitize an entire area by using an	Lidocaine (Xylocaine)	Immediate onset; Lasts about 1-2 hours (with epinephrine) or 1 hour without
		Bupivicaine	Onset 20 min;

			Duration 4-6 hours
		Mepivicaine	Immediate onset; Duration 90-180 minutes
		Novocaine	Topical (mucous membranes) and injectable forms; Immediate onset; Duration 1 hour
		Ophthane	For ophthalmic use

Determining the best pain reliever depends in part, on the type of pain. The two main categories are acute pain and chronic pain. Acute pain is usually temporary and results from something specific, such as a surgery, injuries or infections. Chronic pain is any pain that lasts more than three months and may disrupt daily life. Sometimes chronic pain is just a nagging discomfort, but it can flare up into severe pain. Narcotic analgesics are used to treat some kinds of serious, chronic pain. But for most types of chronic pain, a combination of non-narcotic medication and life style changes is recommended.

Side effects are one reason to be careful about frequent or long-term use of pain relievers.Narcotic analgesics are very effective, but can cause addiction. Aspirin and ibuprofen can irritate the stomach. Acetaminophen (Tylenol, Panadol) does not produce the side effects that aspirin, ibuprofen do, but high doses can cause liver damage, especially in people who drink alcohol regularly. Some pain relievers contain caffeine, which enhances their effectiveness. Taking these drugs near bedtime can interfere with sleep. Anyone who gets edgy or jittery from caffeine should also be careful about using them during the day.

Pain carrying pathway

When there is tissue damage in the periphery, afferent fibers from the damaged site carry the pain impulses via dorsal root ganglion and terminate in the SubstanciaGelatinosaRolandi (SGR) situated at the tip of the dorsal horn. The first neuron ends here.

The second neuron arises from the SGR, crosses to the opposite side and constitutes anterior and lateral spinothalamic tract (STT).

There are 2 groups of fibres in the STT

Direct spinothalamic system—these fibers terminates in the ventral posterolateral nucleus of the thalamus. Next order neuron arises from there and terminates in the post-central gyrus of the cerebral cortex.

Spino-reticular thalamic system—these fibers arising from SGR terminate in the intermediate relaying center of the brain stem. Next order neuron arises from here and to terminate in the thalamus, then to the cortex.

Mechanism of action of narcotic analgesic

All the opioid analgesics bind with selective endogenous opioid receptors and produces action. They are all G-protein linked (2^{nd}messenger system). They produce action by 2 well known mechanisms.

1. They either close voltage gated Ca^{+2} channel presynaptically and decreases the release of excitatory neurotransmitters, such as substance –P, Ach, Glutamate etc.
2. They may hyperpolarize or inhibit post-synaptic membrane by opening of K^{+} channel.

Mechanism of opioid analgesic

Pain consists of both sensory and affective (emotional) components. Opioid can change both components by,

i. Opioid agonists inhibit the release of excitatory neurotransmitter from the primary afferents of the spinal cord and directly inhibit dorsal horn pain transmission neuron of the spinal cord.

ii. It also directly inhibits the sites concerned with pain modulation. Eg:-clostrum.

iii. ↑ Pain threshold.

iv. It also releases endogenous opioid peptide.

Description

Pain has been classified as "productive" and "non-productive." While this distinction has no physiologic meaning, it may serve as a guide to treatment. Productive pain has been described as a warning of injury and so may be both an indication of need for treatment and a guide to diagnosis. Non-productive pain by definition serves no purpose either as a warning or diagnostic tool.

Although pain syndromes may be dissimilar, the common factor is a sensory pathway from the affected organ to the brain. Analgesics work at the level of the nerves, either by blocking the signal from the peripheral nervous system or by distorting the interpretation by the central nervous system. Selection of an appropriate analgesic is based on consideration of the risk-benefit factors of each class of drugs, based on type of pain, severity of pain, and risk of adverse effects. Traditionally, pain has been divided into two classes, acute and chronic, although severity and projected patient survival are other factors that must be considered in drug selection.

Acute pain

Acute pain is self-limiting in duration and includes post-operative pain, pain of injury and childbirth. Because pain of these types is expected to be short term, the long-term side effects of analgesic therapy may routinely be ignored. Thus, these patients may safely be treated with narcotic analgesics without concern for their addictive potential, or NSAIDs with only limited concern for their ulcerogenic

(ulcer-causing) risks. Drugs and doses should be adjusted based on observation of healing rate, switching patients from high to low doses and from narcotic analgesics to non-narcotics when circumstances permit.

An important consideration of pain management in severe pain is that patients should not be subject to the return of pain. Analgesics should be dosed adequately to assure that the pain is at least tolerable and frequently enough to avoid the anxiety that accompanies the anticipated return of pain. Generally analgesics should not be dosed on an as-needed basis but should be administered often enough to assure constant blood levels of analgesic. This applies to both the narcotic and non-narcotic analgesics.

Chronic pain

Chronic pain, pain lasting over three months and severe enough to impair function, is more difficult to treat, since the anticipated side effects of the analgesics are more difficult to manage. In the case of narcotic analgesics this means the **addiction** potential, as well as respiratory depression and **constipation**. For the NSAIDs, the risk of gastric ulcers may be dose limiting. While some classes of drugs, such as the narcotic agonist / antagonist drugs buprenorphine, nalbuphine and pentazocineand the selective COX-2 inhibitors celecoxib and rofecoxib represent advances in reduction of adverse effects, they are still not fully suitable for long-term management of severe pain.

2. PLANT PROFILE

2.1 DESCRIPTION OF THE PLANT

Kingdom	-	Plantae
Subkingdom	-	Angiosperms
Division	-	Magnoliophyta
Order	-	Magnoliales
Family	-	Annonaceae
Subfamily	-	Maloideae
Genus	-	Annona
Species	-	A. squamosa L.
Synonyms	-	Annona asiatica L.
	-	Annona cinerea Dunal
	-	Guanabanus squamosus(L)

VERNACULAR NAMES

- English : Custard-apple, sugar-apple, sweetsop
- Hindi : Shareefa
- Malayalam : Aathappazham
- Tamil : Seetha pazham
- Telugu : Seetha phalam

DESCRIPTION

Annona squamosa is a small, semi-deciduous tree, 3-7 m in height, with a broad, open crown or irregularly spreading branches; bark light brown with visible leaf scars and smoothish to slightly fissured into plates; inner bark light yellow and slightly bitter; twigs become brown with light brown dots(lenticels).

LEAVES

Leaves occur singly, 6-17 x 3-6 cm, lanceolate or oblong lanceolate, pale green on both surfaces and glabrate or nearly so; sides sometimes slightly unequal; edges without teeth, inconspicuously hairy, at least when young, minutely dotted on examination with a lens; thin, dull green to dark green on top surface, and pale blue-green and covered with bloom on underside; apex short or long pointed; base short pointed or rounded; petioles 0.6-1.3 cm long, green, sparsely pubescent.

FLOWERS

Flowers greenish-yellow, fragrant, on slender hairy stalks, produced singly or in short lateral clusters about 2.5 cm long, 2-4 flowers but not at the base of the leaves; sepals pointed, hairy, green, about 16 mm long; 3 outer petals oblong, thick and rounded at the tips, fleshy, 1.6-2.5 cm long,0.6 cm wide, yellow-green, slightly hairy, inside light yellow and keeled with a purplish or reddish spot at the thin, enlarged base; inner petals 3 minute, ovate, pointed scales; stamens very numerous, crowded, white and less than 16 mm long; ovary light green, styles white, crowded on the raised axis.

FRUIT

The aggregate fruit formed from the numerous pistils of a flower, which are loosely united, is soft and distinct from other species of the genus.Each pistil forms a separate tubercle, mostly 1.3-1.9 cm long and 0.6-1.3cm wide. Fruit is round, heart shaped, ovate or conical, 5-10 cm in diameter, with many round protuberances; greenish-yellow when ripe, witha white, powdery bloom; the pulp is white, edible and sweetly aromatic; ineach carpel is embedded a seed, oblong, shiny and smooth, blackish ordark brown, 1.3-1.6 cm long, numerous.

GEOGRAPHIC SPREAD

Annona squamosa native range extends from Africa to Asia including India, Philippines, China, Indonesia, Malaysia, Thailand, and Vietnam, eastern Papua, New Guinea and Northern Territories (Australia) (PIER, 2003). Introduction of it in

the Federated States of Micronesia, Fiji, Guam, Saipan, Hawaii islands and Marshall Islands has been documented.

ECOLOGY

A. *squamosa* is distributed throughout the tropics and is preeminently a desert fruit. Trees do well in hot and relatively dry climates such as those of the low-lying interior plains of many tropical countries.A. squamosa has the reputation, particularly in India, of being a hardy, rought-resistant crop. This is only partly correct. Although the rest period and leaf fall enable the tree to bridge a severe dry season, it requires adequate moisture during the growing season, responding well to supplementary irrigation. The importance of moisture is borne out by the fact that in India as well as Southeast Asia, fruit set is largely limited to the onset of the rains, notwithstanding the prolonged flowering season. Trees are common on the dry coast of Puerto Rico, and also grow in Vieques, St Croix, St Thomas, StJohn, Tortola, and Virgin Gorda.

PARTS USED

Annona seeds, Annona leaves, Annona bark, twigs.

ACTIVE INGREDIENTS

The active ingredients in *Annona squamosa* includeglycosides,alkaloids, saponins, flavonoids, tannins,carbohydrates, proteins, phenolic compounds, phytosterols and amino acids.

PHARMACOLOGICAL EFFECTS

Folkloric record reports its use as an insecticide and an anti-tumor agent, anti-diabetic, anti-oxidant and anti-lipidimic activity, anti-inflammatory activities due to presence of cyclic peptides. In addition, the crushed leaves are sniffed to overcome hysteria and fainting spells, they are also applied on ulcers and wounds, and a leaf decoction is taken in case of dysentery.

MEDICINAL USES

- The bark of custard apple tree can be used to stop diarrhea in children and adults. In addition, the plant is effective to treat diabetes.
- Its fruit is used to make a hair tonic in some parts of India.
- The plant bears some amazing medicinal qualities, like serving as an insecticide, anti-ovulatory and abortificient.
- The grounded seeds can be applied on hair, to get rid of lice. However, make sure that it does not come in contact with ice or else, it can irritate the eye, leading to blindness.
- Custard apple can treat burning sensation, as it is an effective coolant.
- It is used to produce sugar wine apple and is the perfect plant for indoors.
- The crushed leaves of the tree are used to treat hysteria (fearful state of mind) and fainting spells.
- The treatment of ulcer, wound, dysentery and other ailments is also done by its concentrated leaf extract (in which the leaf is boiled and its essence is extracted).
- The good part of custard apple tree is that its remains disease free most of the time; however, it is susceptible to fungus and wilt.
- Ants can create problems for the fruit, by producing mealy bugs on it.
- The roots of the tree are quite powerful and can cause abortions; hence, expecting mothers should take care while eating the herb.

3. LITERATURE REVIEW

- **Mohamed Saleem *et al.*, 2008** investigated the hepatoprotective effect of alcoholic and water extract of *Annonasquamosa* (custard apple) hepatotoxic animals with a view to explore its use for the treatment of hepatotoxicity in human. These extracts were used to study the Hepatoprotective effect in isoniazid + rifampicin induced hepatotoxic model. There was a significant decrease in total bilirubin accompanied by significant increase in the level of total protein and also significant decrease in ALP, AST, ALT and γ-GT in treatment group as compared to the hepatotoxic group. In the histopathological study the hepatotoxic group showed hepatocyticnecrosis and inflammation in the centrilobular region with portal triaditis. The treatment group showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal. It should be concluded that the extracts of *Annonasquamosa* were not able to revert completely hepatic injury induced by isoniazid + rifampicin, but it could limit the effect of these drugs in liver. The effect of extracts compared with standard drug silymarin.

- **SharmaAbhishek *et al.*, 2009** investigated the macroscopically, microscopically& preliminary phytochemical studies on the leaf of *Annonasquamosa*Linn., All the parameters were studied according to the WHO &Pharmacopoeial guidelines. The qualitative phytochemical fingerprint of the methonalic extract revealed the presence of alkaloids, terpenoids, phenolics, fats and waxes. The aqueous leaf slurry was found to be safe at the dose level of 2g/kg body weight of mice.

- **Aditya V *et al.*, 2010** investigatedthe separation and identification of the active compounds against head lice from the ethyl acetate extract of *Annonasquamosa* seed. Chromatographic and spectroscopic techniques revealed that two major compounds of the hexane seed extract were oleic acid and triglyceride with one oleate ester. The yields of these compounds were 13.88% and 7.70% dry weight, respectively. The compounds were tested in vitro against head lice, comparing to the crude ethyl acetate extract of the seed. The triglyceride with one oleate ester and the crude ethyl acetate extract diluted with coconut oil 1:1. These compounds were found to kill all tested head lice in 10 and 31 minutes, respectively. The triglyceride

ester can be used as a marker for quantitative analysis of the active compound for quality control of the raw material A. *squamosa* seed and its extract. This finding will be useful for quality assessment and the chemical stability of the anti-head lice preparation from this plant.

- **Pardhasaradhi *et al.*, 2005** investigatedthepesticidal,parasiticidal,anti-microbial, cell growth inhibitory activities. In this study, organic and aqueous extracts from thedefatted seeds of *Annonasquamosa*(custard apple) were tested on different human tumor cell lines for antitumouralactivity. While organic and aqueous extracts induced apoptosis in MCF-7 and K-562 cells, they failed todo so in COLO-205 cells. Treatment of MCF-7 and K-562 cells with organic and aqueous extracts resulted in nuclear condensation, DNA fragmentation, induction of reactive oxygen species (ROS) generation and reduced intracellular glutathione levels. In addition down regulation of Bcl-2 and PS externalization by Annexin-V stainingsuggested induction of apoptosis in MCF-7 and K-562 cells by both the extracts through oxidative stress. Onthe contrary, COLO-205 cells showed only PS externalization but no change in ROS and glutathione levels. Theseobservations suggest that the induction of apoptosis by *A. squamosa*extracts can be selective for certain types of cancerous cells.

- **RakeshRanjan *et al.*, 2008** investigatedthephytochemical investigations of *Annonasquamosa*seeds have led to the isolation of three lignans consisting of coumarin moiety, Cleomiscosin A, Cleomiscosin B and Cleomiscosin C. Their structures were arrived at by detailedspectroscopic analysis. Cleomiscosin A and Cleomiscosin B are position isomer.

- **Nighat Begum *et al.*, 2010** investigated the crude ethanol extracts of *Calotropisprocera*and *Annonasquamosa*leaves have beenscreened for their against *Muscadomestica*. The third instar larvae of housefly were treated with thedifferent concentrations of both the extracts by dipping method for 48 h. The LC50 values of the extracts of *C. procera*and*A. squamosa*leaves were found to be 282.5 and 550 mgl-1, respectively. The phytochemical analysis of these extracts suggested presence of alkaloids as the major component. The larvae were exposed to 5 and

10% concentrations of the LC50 value of each extract along with their control sets to evaluate their effect on metamorphosis, nucleic acid and protein content in different developmental stages. The leaf extract of *C. procera*was found to be more active in terms of insecticidal potential. The data indicate that the leaf extracts of these plants may be utilized as the probable candidates for the development of bio insecticides to control the population of *Muscadomestica*as safer and economic alternativesto the synthetic insecticides.

- **Kaleem M *et al.*, 2008** investigated the possible therapeutic effects of *Annonasquamosa* (*A.squamosa)* extract on certain biochemicalmarkers in streptozotocin (STZ) –induceddiabetes mellitus in rats.

- **M. Lebrini *et al.*, 2010** investigated the *Annonasquamosa*plant have been studied as possible corrosioninhibitor for C38 steel in molar hydrochloric acid (1 M HCl). Potentiodynamic polarization and ACimpedance methods have been used. The corrosion inhibition efficiency increases on increasing plantextract concentration. Polarization studies showed that *Annonasquamosa* extract was mixed-typeinhibitor in 1 M HCl. The inhibition efficiency of *Annonasquamosa* extract was temperaturedependentand its addition led to an increase of the activation corrosion energy revealing a physicaladsorption between the extract and the metal surface. The adsorption of the *Annonasquamosa*extract followed Langmuir's adsorption isotherm. The inhibitive effect of *Annonasquamosa* is ascribed to thepresence of organic compounds in the extract. The examined extract is considered as non-cytotoxicsubstance.

- **Marta Souza *et al.*, 2007** investigated the anthelmintic activity against Haemonchuscontortus, the main nematodeof sheep and goat in Northeastern Brazil. A compound 1 was isolated from ethyl acetate extract and inhibited theegg hatching of H. contortus at 25 mg ml^{-1}. The structure of 1 was determined as a C_{37} trihydroxy adjacent bis-tetrahydrofuranacetogenin based on spectroscopic analysis.

- **Pardhasaradhi *et al.*, 2004** investigated the rat histiocytictumor cell line,AK-5.Both the extracts caused significantApoptotictumor cell death with enhanced capase-3

activity, down regulation of anti-apoptotic genes Bcl_{XL}.In addition, DNA fragmentation and annexine-v staining,confirmed that the extracts induced apoptosis in tumor cell through the oxidativestress.Aqueous extracts of *Annona squamosa* seeds possessed significant anti-tumor activity in vivo against AK-5 tumor.

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- **Audrey Leatemia *et al.*, 2004** investigated the inhibition of larval growth against thepolyphagouslepidopteranSpodopteralitura (Noctuidae). Extracts of *A.squamosa* weresignificantly more active (20-fold) than those of *A.muricata.A. squamosa* collected fromNamlea yielded the extracts with the greatest inhibitory activity. There were significantdifferences among locations for both *A. squamosa* and *A. muricata* but not for *L. domesticum*and *S. koetjape*. Extracts of A. *squamosa*, collected from Namlea, inhibited larval growth in a dose-dependent manner, with a dietary EC_{50}(effective concentration to inhibit growth by 50% relative to controls) of 191.7 ppm fresh weight. Extracts of A. *squamosa* collectedfrom individual trees in Namlea also varied in growth inhibitory effect against S. litura andTrichoplusiani larvae. This species is a candidate for development of a botanical insecticidefor local use in Indonesia.

- **Mohamed Saleem *et al.*, 2011** investigated the protective effect of methonalic extract of *Annonasquamosa* onisoniazid-rifampicin-induced hepatotoxicity in rats. Rats were divided into five different groups (n=6), group 1served as a control, Group 2 received isoniazid (100 mg/kg, i.p.) and co-administered with rifampicin (100mg/kg, i.p.), in sterile water, group 3 and 4 served as extract treatment groups and received 250 & 500 mg/kgbw, p.omethonalic extract of *Annonasquamosa* and group 5 served as standard group and received Silymarin 2.5 mg/kg bw, p.o. All the treatment protocols followed 21 days and after rats were sacrificed blood and liver were used for biochemical and histological studies, respectively. Administration of isoniazid and rifampicincaused a significant elevation in the levels of liver marker enzymes and thiobarbituric acid reactive substances (TBARS, oxidative stress markers) in experimental rats. Administration of methonalic extracts of *Annonasquamosa* significantly prevented isoniazid-rifampicin-induced elevation in the levels of serum diagnostic livermarker enzymes (alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase(ALP) and gamma

glutamate trans peptidase (γ-GT)), serum bilirubin, and TBARS level in experimental groups of rats. Moreover, total protein and reduced glutathione (GSH) levels were significantly increased in treatment group. The effect of extract was compared with a standard drug, silymarin. The changes in biochemical parameters were supported by histological profile. It is to be concluded that the methonalic extract of *Annonasquamosa* protects against isoniazid and rifampicin-induced oxidative liver injury in rats.

- **NehaPandey *et al.*, 2011** investigated the antioxidant, anti diabetic, hepatoprotective, cytotoxic activity, gene toxicity, antitumor activity, antilice activities of aqueous and methonalic extracts of *Annonasquamosa* leaves and roots. It is related to contain alkaloids,carbohydrates, fixed oils, tannins & phenolic compounds.

- **Tylor Johns *et al.*, 2011** investigated the antimalarial activity of ethonalic extract of *Annonasquamosa* bark, which is traditionally used in diseases including infections associated with malarial parasites. N-Nitrosoxylopine, roemerolidine and Duguevalline were isolated from the extract of bark. All compounds showed moderate activity against chloroquine sensitive strain (D10) and a chloroquine resistant strain (Dd2) of Plasmodium falciparum with IC_{50} values ranging between 7.8 and 34.2μM/mL. N-Nitrosoxylopine also showed cytotoxicity in MTT assay while no cytotoxicity was observed for other two compounds.

- **Dinesh K. Yadav *et al.*, 2011** investigated anticancer activity of ethonalic, chloroform, butanalic and aqueous extracts of *Annonasquamosa* twigs, resulted in isolation and identification of twelve known (1-12) compounds among them one 1-(4-β-D-glucopyranosyloxyphenyl)-2-(β-D-glucopyranosyloxy)-ethane(11) is synthetically known but first time isolated from natural sources. Their structures were elucidated using 1D and 2D NMR spectroscopic analysis. The isolated compounds (2-8, 11) were evaluated for H+ K+-ATPase activity. Three of these compounds (+)-O-methylarmepavine, N-methylcorydaldine,isocorydine showed promisinganti-secretory activity. Activity of these compounds, comparable to standard drug omeprazole is novel to our finding. Moreover, there is no information

accessible regarding the pharmacological effect of *Annonasquamosa* on the gastrointestinal system. This study is the first of its kind to show significant anti-ulcer effect of *Annonasquamosa*. Present study aimed to evaluate the gastro protective effect of *Annonasquamosa* (AS) and to identify its active constituents. Anti-ulcer activity was evaluated against cold restraint (CRU), pyloric ligation (PL), aspirin (ASP), alcohol (AL) induced gastric ulcer and histamine (HA) induced duodenal ulcer model and further confirmed through in vitro assay of H+ K+-ATPase activity and plasma gastrin level. AS and its chloroform and hexane fraction attenuated ulcer formation in CRU, PL, HA model and displayed anti-secretory activity in vivo through reduced free, total acidity and pepsin in PL, confirmed by in vitro inhibition of H+ K+-ATPase activity with corresponding decrease in plasma gastrin level. Cytoprotection of AS was apparent with protection in AL, ASP models and enhanced mucin level in PL.

- **L. G. Matos *et al.*, 2002** investigated acetic acid-induced abdominal writhing, the tail flick test and carrageenan-induced peritonitis were used to study the analgesic and anti-inflammatory activity of the crude ethanolic extract from *Spirantheraodoratissima*roots. Pentobarbital-induced sleeping time was used to study the central depressant effect of the extract. Theethanolic extract caused a dose dependent inhibition of acetic acid-induced abdominal writhing and leukocyte migration, and produced a significant, dose-related increase in the duration of sleep. The results suggest that *Spirantheraodoratissima* roots contain compounds with anti-inflammatory and central depressant actions.

- **Paul V Tan *et al*., 2002** evaluated the anti-ulcerogenic effects of the leaf methanol extract of *Ocimum suave* using four ulcer models in Wistar rats. Administration of extract to the rats showed dose dependent reduction in gastric ulcer in all four models, accompanied by significant increase in the gastric mucus production. The extract dose 500mg/kg in pylorus ligation model inhibited the gastric lesion formation and less effect on gastric secretion as observed with the control. In ethanol induced model, the dose 250mg/kg showed complete inhibition of gastric lesion. During high gastric acidic environment the effect of extract on mucus secretion was not much significant.

- **Akilandeswari *et al.*, 2010** evaluated the screening of gastric antiulcer activity of sida acute using aspirin plus pylorus ligation, aspirin induced and ethanol induced ulcer model. The normal control exhibited very severe ulceration in aspirin plus pylorus and aspirin. Hence aspirin proved to be most potent in gastric ulcer induction. In aspirin (300mg/kg) plus pylorus model the statistical data 100mg/kg shows significant when compared to famotidine group. The group 200mg/kg showed significant activity with famotidine. In ethanol model the statistical data 100mgkg shows significant when compared to famotidine group. The flavonoids have been reported to possess significant antiulcer activity in various experimental model.

- **Jaikumar *et al.*,** investigated the antiulcer activity of methonalic extract of *Jatrophacurcus* on aspirin plus pylorus ligation induced gastric lesion in Wistar strain rats. In the present study, methonalic extract of jc have been shown to posses antiulcer activity against experimentally induced ulcer model, methonalic extract significantly reduced ($p<o.o5$) the acid secretory parameters i.e free and total acidity, gastric volume and ulcer index. Aspirin plus pylorus group show the ulcerated mucosa with hemorrhage and discontinuity of lining epithelium while cut showed the normal mucosa with mild hyperplasia and mild edematous sub mucosa, compared to ranitidinetreated group which show the normal mucosa with no ulcer.

- **Kannappan *et al.*, 2008** Studied the effect of methonalic extract of leaves of *Jatrophacurcas Linn* on pylorus ligation and aspirin induced ulcer in Wistar rats. Administration of extract dose (100mg/kg) and ranitidine (50mg/kg) for 5 days to the aspirin plus pylorus ligation rats and its parameter were observed. The extract showed the markedly reduction in the acid parameter like gastric volume, p^H , free acidity and total acidity. Treatment with extract 100 and 200mg/kg showed dose dependent reduction in ulcer index. Histopathological studies revealed that the stomach mucosa showed the protective action of extract against mucosal damage caused by aspirin.

- **Malairajan *et al.*, 2006** evaluated the anti-ulcer activity of *Toonaciliata roemer*against aspirin plus pylorus ligation (ant secretory), ethanol-induced ulcer

and stress induced ulcer in rats. The plant extract markedly showed gastroprotective activity when compared with that of standard drug sucralfate. The extract showed decrease in the ulcer index and significant reduction in gastric volume, free acidity, total acidity.

- **Sunita M *et al..,*** investigated the anti-ulcer activity of *Benincasahispada*(Thunb) fruit. The anti-ulcer activity was evaluated in rats against ethanol-induced gastric mucosal damage, pylorus-ligated gastric models and cold restraint-stress induced gastric ulcers. Many parameters were studied for the models employed in ulcer studies of which ulcer index was common one for all the models. Vascular permeability was evaluated in the ethanol model. Effect of peroxidation, vizmelondialdehyde (MDA) content, superoxide desmutase (SOD) and catalase (CAT) were studied in Cold Restraint Stress model. The results showed significant reduction in ulcer index in all the models and the results were comparable with the Omeprazole-treated group.

- **B Rajkapoor *et al.,*** investigated the effect of alcoholic extract of dried fruits of *Carica papaya* in rats to evaluate the antiulcer activity by using pyloric ligation and aspirin-induced gastric ulcer. The parameters taken to assess antiulcer activity were volume of gastric secretion, free acidity, total acidity and ulcer index. The results indicate that the alcoholic extract significantly ($P<0.001$) decreases the volume of gastric acid secretion, free acidity, total acidity and ulcer index with respect to control.

- **S. Narayan *et al.,*** investigated the protective effect of ambrex, a poly-herbal drug, in ethanol induce gastric mucosal lesions in experimental rats. The response to ambrex was assessed from ulcer index, cell proliferation, histopathological changes and alkaline phosphatase (ALP) activity. Ambrex pre-treatment showed protection against ethanol-induced gastric mucosal damage, a significant reduction in the ulcer index and ALP activity and an increase in DNA content.

- **Madhu *et al.,*** investigated the anti-ulcer activity of *Wrightiatinctora(Roxb).* The antiulcer activity of the Wrightiatinctoriamethonalic extract and Wrightiatinctoria

70% ethanolic extract were compared with carboxy methyl cellulose (CMC), pylorus control, Aspirin and standard famotidine which was evaluated by employing aspirin plus pylorus ligation induced ulcer model. The Biochemical parameters like volume of gastric juice secretion, pH, free acidity, total acidity, ulcer index and percentage inhibition were studied at the concentration of 200 mg/kg body weight. The results showed significant gastro-protective effect when compared to the standard drug Famotidine.

4. AIM AND OBJECTIVE

Medicinal plants have been in India for centuries as a therapeutic source for treating wide variety of ailments and have been found to be of immense global importance. It is estimated by World Health Organization that, 80% of the world population must rely on traditional medicines for health care: these traditional medicines are mainly plant based. Most of the studies demonstrate the importance of natural products in drug discovery. The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs. The selection of plant was made on the basis of its availability, therapeutic value and degree of research work which is not done.

Peptic ulcer disease(PUD) is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy. It includes number of drugs such as proton pump inhibitors and H2 receptor antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapse, side effects, and drug interaction. This has been the rational for the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse. Drug of plant origin are gaining popularity and are being investigated for a number of disorders, including peptic ulcer.

Peptic ulcer occurs due to an imbalance between aggressive (acid, pepsin) and defensive (gastric mucosal barrier) factors of gastric mucosa. Local mechanism implicated in mucosal hydrophobicity, rapid epithelial cell renewal and rich mucosal blood flow. PGE2 and PGI2 are predominant prostaglandins synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. The treatment of peptic ulcer is directed against either reduction of aggressive factors or enhancement of mucosal defense of stomach and duodenum with cytoprotective agents.

In India, PUD is common. In the Indian Pharmaceutical industry, antacids and antiulcer drugs share 6.2 billion rupees and occupy 4.3% of the market share. Today, there are two main approaches for treating peptic ulcer. The first deals with

reducing the production of gastric acid and second with re-enforcing gastric mucosal barrier. (Dharmani P et al., 2006)

Analgesia may be loss of sensation of pain that result from an interruption in the nervous system pathway between sense organ and brain. Different form of sensation (touch, temperature and pain) stimulating an area of skin travels to the spinal cord by different nerves fibres in the same nerve bundle.

According to the current unifying concept of NSAIDS action, during pain, inflammation, fever, arachidonic acid is liberated from phospholipase fraction of the cell membrane; arachidonic acid is then converted to prostaglandins via cyclo-oxygenase (COX 1 & 2) pathway. COX-1 is constitutively present in stomach, kidney and blood vessels whereas COX-2 is inducible in activated leucocytes and other inflammatory cells. It is postulated that PGs sensitize blood vessels to the effects of mediators such as 5-HT, bradykinin and histamine that increase the permeability. PGs particularly PGE and PGI produce hyperalgesia associated with inflammation. Pain can be constant (chronic) or fleeting and come and go (acute). There are many herbs that are useful and effective for pain relief many are safe for everyone but some should be avoided.

OBJECTIVES OF THE STUDY

The objectives of the present study are-

- Exploring the traditional medicines with proper chemical and pharmacological profiles.
- Extraction of *Annona squamosa* leaf by different solvents.
- To conduct systematic phytochemical investigation of *Annona squamosa*.
- To evaluate the antiulcer activity in rats.
- To evaluate the analgesic activity in mice.

5. PLAN OF WORK

PHYTOCHEMICAL STUDIES

1. Collection of plant (*Annona squamosa*) leaves and shade dried.
2. Extraction of *Annona squamosa* leaves powder using ethanol in soxhlet apparatus for 72 hrs.
3. Phytochemical investigation of ethanolic extract of *Annona squamosa* leaf.

PHARMACOLOGICAL STUDIES

1. Evaluation of anti-ulcer activity of *Annona squamosa* by

- Aspirin induced ulcer model
- Pylorus ligation model

1.1 Evaluation of analgesic activity of *Annona squamosa* by

- Acetic acid induced writhing in mice

1.2 Determination of following parameters

- Ulcer index
- Percentage inhibition
- Gastric volume
- pH of gastric juice
- Total acidity
- Free acidity

1.3 Histopathological studies

6. MATERIALS AND METHODS

PLANT MATERIAL

The fresh leaves of *Annona Squamosa* L were collected from local area at komarapalayam, Tamilnadu. The material was taxonomically identified, confirmed and authenticated by **Botanical survey of India (BSI) at TN agricultural university, Coimbatore**. With authentication no **BSI/SRC/5/23/2011-12/Tech.-1201** and the voucher specimen was retained in our laboratory for further reference. The collected leaves were shade dried and the dried material was crushed to coarse powder with mechanical grinder. The powder was stored in air-tight container which was used for extraction.

6.1. PHYTOCHEMICAL STUDIES

6.1.1 EXTRACTION OF PLANT MATERIAL

- **Ethanolic extraction**

About 300 gm of air dried powdered material was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 18 hours till the solvent became colourless. At the end of the extraction process the marc was taken out and it was dried. After drying, the powdered marc was weighed & again packed and extracted with ethanol for another 72 hours till it became colourless. After that extract was concentrated by distillation. The final solution was evaporated, to obtain a syrupy greenish mass.

6.1.2. PRELIMINARY PHYTOCHEMICAL ANALYSIS

The plant may be subjected to preliminary phytochemical screening for detection of various bioactive chemical constituents. The important steps involved in the phytochemical screening of a plant include extraction of constituents using suitable solvents followed by screening with various chemical tests.

In the process of phytochemical screening, the crude extracts or isolated constituents are subjected to qualitative and quantitative chemical analyses. Qualitative chemical analysis includes the determination of nature of the

constituents in an extract or its fractions which lead to the isolation of the active lead compound. Quantitative chemical analysis includes the determination of the purity of isolated substances or group of substances in a mixture by finger printing and different analytical techniques.

- **Qualitative tests**

A) Test for carbohydrates

1. Molisch Test

It consists of treating the compounds of α-naphthol and concentrated sulphuric acid along the sides of the test tube. Purple color or reddish violet color at the junction between two liquids.

2. Fehling's Test

Equal quantity of Fehling's solution A and B is added. Heat gently, brick red precipitate is obtained.

3. Benedict's test

To the 5ml of Benedict's reagent, add 8 drops of solution under examination. Mix well, boiling the mixture vigorously for two minutes and then cool. Red precipitate is obtained.

4. Barfoed's test

To the 5ml of the Barfoed's solution add 0.5ml of solution under examination, heat to boiling, formation of red precipitate of copper oxide is obtained.

B) Test for Alkaloids

1. **Dragendroff's Test**

To the extract, add 1ml of Dragendroff's reagent Orange red precipitate is produced.

2. **Wagner's test**

To the extract add Wagner reagent. Reddish brown precipitate is produced.

3. Mayer's Test

To the extract add 1ml or 2ml of Mayer's reagent. Dull white precipitate is produced.

4. **Hager's Test**

To the extract add 3ml of Hager's reagent, yellow precipitate is produced.

C) Test for Steroids and Sterols

1. **Libermann Burchard test**

Dissolve the test sample in 2ml of chloroform in a dry test tube. Now add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green in color.

2. **Salkowski test**

Dissolve the sample of test solution in chloroform and add equal volume of conc. sulphuric acid. Bluish red cherry red and purple color is noted in chloroform layer, whereas acid assumes marked green fluorescence.

D) Test for Glycosides

1. **Legal's test**

Sample is dissolved in pyridine; sodium nitropruside solution is added to it and made alkaline. Pink red color is produced.

2. **Baljet test**

To the drug sample, sodium picrate solution is added. Yellow to orange color is produced.

3. **Borntrager test**

Add a few ml of dilute sulphuric acid to the test solution. Boil, filter and extract the filtrate with ether or chloroform. Then organic layer is separated to which ammonia is added, pink, red or violet colour is produced in organic layer.

4. **Killer Killani test**

Sample is dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid reddish brown color is produced which gradually becomes blue.

E) Test for Saponins

Foam test

About 1ml of alcoholic sample is diluted separately with distilled water to 20ml, and shaken in graduated cylinder for 15 minutes.1 cm layer of foam indicates the presence of Saponins.

F) Test for Flavonoids

Shinoda test

To the sample, magnesium turnings and then concentrated hydrochloric acid is added. Red color is produced.

Lead acetate test

The extracts were treated with few drops of lead acetate solution, formation of yellow

Precipitate indicates the presence of flavonoids.

Alkaline reagent test

The extracts were treated with few drops of sodium hydroxide separately. Formation of intense Yellow color, which becomes colorless on addition of few drops of dilute acid, indicates the presence of flavonoids.

G) Test for Triterpenoid

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution is added. Pink colour is produced which indicates the presence of triterpenoids.

H) Test for Protein and Amino acid

1**. Biuret test**

Add 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate to the extract, a violet colour indicates the presence of proteins.

2. **Ninhydrin test**

Add 2 drops of freshly prepared 0.2% ninhydrin reagent to the extract and heat. A blue colour develops indicating the presence of proteins, peptides or amino acids.

3. **Xanthoprotein test**

To the extract, add 20% of sodium hydroxide or ammonia. Orange colour indicates presence of aromatic amino acid. **(Evans W.C 1996, Kandelwal, 2004.)**

6.2 ACUTE ORAL TOXICITY STUDIES

Animals

Swiss albino mice of female sex weighing 20-25gms were used for the study. The animals were obtained from Agricultural University, Manuthy, Trissur and were housed in polypropylene cages. The animals were maintained under standard laboratory conditions ($25^0 \pm 2^0$C; 12hr light and dark cycle). The animals were fed with standard diet and water *ad libitum*. Ethical clearance (for handling of animals and the procedures used in study) was obtained from the Institutional Animal Ethical Committee before performing the study on animals.

Acute toxicity Test

Acute oral toxicity study for ethanol extract of *Annona squamosa* leaves was carried out as per OECD guideline 425 (Up and Down procedure). The test procedure minimizes the number of animals required to estimate the acute oral toxicity. The test allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

Animals were fasted (food but not water was with held overnight) prior to dosing. The fasted body weight of each animal was determined and the dose was calculated according to the body weight.

6.3 PHARMACOLOGICAL EVALUATION

6.3.1 EVALUATION OF ANTI-ULCER ACTIVITY

a) Animal study

Male albino-Wistar rats, weighing 150-200g were used in the present study.

Housing and feeding condition

All the rats were kept at room temperature (22±30°C) in the animal house. All the animals were housed and treated as per the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food and were acclimatized to standard laboratory conditions of temperature (22±30°) and maintained an 12:12 hr light: dark cycle. They were provided with regular rat chow and distilled water *ad libitum.* All the experimental procedures were performed on animal after approval from the ethics committee and in accordance with the recommendations for the proper care and use of laboratory animals.

Extract

Ethanolic extract of *Annona squamosa* leaves.

Standard drug used

- Omeprazole (20mg/kg)
- Acetic acid (1%v/v)
- Pentazocine (30mg/kg)

Drug omeprazole and the extract of *Annona squamosa* were suspended in 0.5% CMC.

b) Anti-ulcer activity

The anti-ulcerogenic potential of the leaf ethanol extract of *Annona squamosa* was investigated by three different models in experimental Wistar rats.

1. Aspirin induced ulcer (Anti-secretary mechanism).

2. Pylorus ligation. (Anti-secretary mechanism).

Two models were used to elucidate the possible mechanism of action of the ethanolic extract of *Annona squamosa.* The respective standard drug (Omeprazole 20mg/kg,) was used for the particular experimental models.

2. EXPERIMENTAL PROCEDURES

a) Aspirin induced ulcer

Male albino-Wistar rats were divided in to four groups as mentioned above of six animals per group and animals were fasted for 24 hrs prior to the experiment in perforated steel cages to avoid coprophagy.

Group I - control.

Group II - received 50mg/kg, p.o ethanol extract of *Annona squamosa* leaves.

Group III - received 100mg/kg, p.o ethanol extract of *Annona squamosa* leaves.

Group IV - received 20mg/kg, p.o Omeprazole as standard.

Group was kept as control without any treatment. One hour after the drug treatment, the animals were treated with aspirin 200mg/kg by p.o, to induce ulcers. The animals were sacrificed after 4hrs and stomach was opened and percentage inhibition of ulcer was determined. **(Kannappan *et al.*, 2008, Panda *et al.*, 1993, Parmar NS *et al.*, 1991, Pati K.S. *et al.*, 2008).**

b) Pylorus ligation model

The animals were divided in to four groups of six animals each as mentioned above.

Group I - received 1% CMC (1.0ml/kg p.o) as vehicle control

Group II - received 20mg/kg, p.o Omeprazole as standard

Group III - received 50mg/kg, p.o ethanol extract of *Annona squamosa* leaves.

Group IV - received 100mg/kg, p.o ethanol extract of *Annona squamosa* leaves.

Animals in all the groups were fasted for 36 h after the respective assigned treatment and were anaesthetized with anaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process and pylorus portion of stomach was lifted out and ligated. Precaution was taken to avoid traction to the blood supply. The stomach was sutured with interrupted sutures. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. Four hours after the pyloric ligation, the animals were sacrificed by an excess dose of ether. The stomach was carefully removed and the gastric contents were collected. The gastric juice was centrifuged at

1000rpm and gastric volume was measured. Free and total acidities of the supernatant were determined by titration with 0.1 N NaOH and expressed as mEq/ L /100 g. The stomach was cut open along the greater curvature and pinned onto a soft board for evaluating the gastric ulcers and to calculate ulcer index. Ulcer scoring is done according to the scale mentioned below.

BIOCHEMICAL PARAMETERS

The stomach was carefully excised keeping oesophagus closed and opened along greater curvature and luminal contents were removed. The gastric contents were collected in a test tube and centrifuged. The gastric contents were analyzed for gastric juice volume, pH, free and total acidity.

MEASUREMENT OF GASTRIC JUICE VOLUME AND PH

Gastric juice was collected from pylorus ligation induced ulcer rats. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min. The volume of supernatant was measured and expressed as ml/100g body weight. The pH of the supernatant was measured using digital pH meter. **(Canmon DC., *et al.*, 1969, Kannappan *et al.*, 2008, Patil K.S. *et al.*, 2008, Paul V. *et al.*, 2000)**

DETERMINATION OF FREE AND TOTAL ACIDITY

An aliquot of 1.0 ml of gastric juice was pipette out in to a 50 ml conical flask and 2/3 drops of Topfers reagent was added to it and titrated with 0.01N NaOH until all traces of the red color disappeared and the color of the solution turned yellowish orange. The volume of 0.01N NaOH was noted which corresponds to free acidity. Then 2/3 drops of phenolphthalein was added and titration was continued until a permanent pink color was developed. The volume of total alkali consumed was noted which corresponds to total acidity. The free acidity and total acidity was determined using the formula and values are expressed as mEq/l 100g. **(Kannappan *et al.*, 2008, Rajkapoor *et al.*, 2002)**

$$\text{Acidity} = \frac{\text{Volume of NaOH X Normality of NaOH X 100}}{0.01} \quad \text{(mEq/L per 100g)}$$

ULCER INDEX (UI)

The mucosa was flushed with saline and stomach was pinned on frog board. The lesion in glandular portion was examined under a 10x magnifying glass and length was measured using a divider and scale and gastric ulcer was scored. Ulcer index of each animal was calculated by adding the values and their mean values were determined. **(Malairajan *et al.*, 2007)**

- 0 – Normal colored stomach
- 0.5 – Red coloration
- 1 – Spot ulceration
- 1.5 – Hemorrhagic streak
- 2 – Ulcers
- 3 – Perforations

PERCENTAGE INHIBITION

Percentage inhibition was calculated using the following formula. (Malairajan *et al.*, 2007)

$$\% \text{ inhibition} = \frac{UI_{\text{ulcer control}} - UI_{\text{ulcer treated}}}{UI_{\text{ulcer control}}} \times 100$$

HISTOPATHOLOGICAL STUDIES

The stomach tissue were removed from the rats and fixed in10% formaline saline for at least 48 hrs. These were then processed routinely and the tissue was embedded in paraffin wax. Histological section were cut at 5-6µm and stained with routine haemotoxylin and eosin. These were examined by a consultant histopathologist.

6.3.9 STATISTICAL STUDIES

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnetts't' test using Graph Pad Prism software. The mean values ± SEM were calculated for each parameter.

6.4 EVALUATION OF ANALGESIC ACTIVITY

6.4.1 ANALGESIC ACTIVITY

The leaf of ethanolic extract of *Annona squamosa* were undertaken for the present study of analgesic activity by

- **Acetic acid induced writhing method**

6.4.2 EXPERIMENTAL PROCEDURE

The analgesic activity was performed on mice, fasted for 12 hrs prior the experiment, but provided water. The animals were divided into four groups of four in each.

Group I - Received acetic acid 1%w/v.

Group II - Received extract 50mg/kg.

Group III - Received extract 100mg/kg.

Group IV - Standard 30mg/kg.

The activity was performed by acetic acid induced writhing method. The ethanolic extract in dose 50 and 100 mg/kg body weight, an acetic acid (1%w/v) is suspended in 1% suspension of carboxy methyl cellulose were injected

intraperitoneally. An aliquot of 0.25 ml of this suspension is injected intraperitoneally in each animal. The animal reacts with a characteristic stretching behavior i.e., a series of constrictions occur that travel along the abdominal wall, sometimes accompanied by turning movements of the body and extension of the hind limbs.

A group of animals (n=4) is used as test group in which prior to acetic acid administration, test drugs are administered orally. The mice are placed individually into glass chambers and number of writhes is recorded for 10 min in each animal.

For scoring, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

Formula for computing percent inhibition is

$$= \frac{\text{Average writhes in control group-writhes in test group}}{\text{Writhes in the control group}} \times 100$$

The time period with the greatest percent of inhibition is considered the peak time

% decrease in activity= 100-(test / standard × 100)

7. RESULTS AND DISCUSSION

The present study shows that the ethanol extract of *Annona squamosa* leaves exerts gastro-protective action and analgesic activity against aspirin induced ulcer model, pylorus ligation mode, aspirin induced ulcer model and acetic acid induced writhing model.

7.1 PRELIMINARY PHYTOCHEMICAL ANALYSIS

Show qualitative chemical tests of leaf extracts of *Annona squamosa*:

Table No: 3

Phytoconstituents	**Extracts**	
	Petroleum ether	**Ethanol**
Carbohydrates	-	+
Glycosides	-	+
Alkaloids	-	+
Phytosteroids	-	+
Flavonoids	-	+
Protein and amino acids	-	+
Saponin	-	+
Phenols & tannins	-	+

\+ = Present - = Absent

The preliminary Phytochemical screening of the extract of *Annona squamosa* leaves showed the presence of carbohydrates, alkaloids, sterols, flavonoids, saponins, tannins and phenolic compounds, Protein and amino acids. The various phytoconstituents present in the extract is shown in the **TABLE 3.**

The phytoconstituents like flavonoids, tannins, terpenoids, phenols and saponins and sterols have been reported in several anti-ulcer literatures as possible gastroprotective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which antiulcerogenic efficacy has been extensively confirmed. **(Borelli F. *et al.*, 2000)**

It is suggested that these compounds will be able to stimulate mucus, bicarbonate and prostaglandin secretion, and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen. (suja pandian et.al., 2002) Tannins are known to "tan" the outermost layer of the mucosa and render it less permeable and more resistant to chemical and mechanical injuries. Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants.**(Berenguer B *et al* .,2005)**

Similarly, the ethanol extract of *Annona squamosa* leaf, showed the presence flavonoids and their alkaloids, phenols and tannins, triterpenoids and saponins. These phytoconstituents present in the extract could be the possible agents involved in the prevention of gastric lesions induced by aspirin, pylorus ligation and ethanol induced models.

Flavonoids are also capable of scavenging the free radicals.

Phenols and Phenolic compounds have potent antioxidant properties **(Berenguer *et al.*, 2005),**

The analgesic activity of *Annona squamosa* may be attributed to the presence of various phytoconstituents such as alkaloids, tannins, carbohydrates, flavonoids.

7.2 PHARMACOLOGICAL STUDIES

7.2.1 ACUTE ORAL TOXICITY STUDIES

The acute oral toxicity of the ethanolic extract of *Annona squamosa* was carried out as per OECD 425-guideline. The acute toxicity studies revealed that LD50>2000mg/kg for the extract. Acute oral Toxicity study (425) observations showed in **Table No 4.**

Table No: 4

RESPIRATORY BLOCKAGE IN NOSTRIL	
Dyspnoea	Nil
Apnoea	Nil
Tachypnea	Nil
Nostril discharge	Nil
MOTOR ACTIVITIES	
Locomotion	Normal
Somnolence	Nil
Loss of righting reflex	Nil
Anaesthesia	Nil
Catalepsy	Nil
Ataxia	Nil
Toe walking	Nil
Prostration	Nil
Fasciculation	Nil
Tremor	Nil
CONVULSION(INVOLUNTRAY CONTRACTION)	
Clonic/tonic/tonic-clonic convulsion	Nil
Asphyxial convulsion	Nil
Opistotones (titanic spasm)	Nil

REFLEXES	
Corneal	Normal
Eyelid closure	Normal
Righting	Normal
Light	Normal
Auditory and sensory	Normal
OCULAR SIGNS	
Lacrimation	Nil
Miosis	Nil
Mydriasis	Nil
Ptosis	Nil
Chromodacryorrhea	
Iritis	Nil
Conjunctivitis	Nil

SALIVATION	
Saliva secretion	Nil
PILOERECTION	
Contraction of erectile tissue	Nil
ANALGESIA	
Decrease in reaction to induced pain	Nil
MUSCLE TONE	
Hypo or hypertonia	Nil
GIT SIGN	
Solid dried / watery stool	Nil
Emesis	Nil
Red urine	Nil
SKIN	
Oedema	Nil
Erythema	Nil

7.2.2 ANTI-ULCER SCREENING

7.2.2 A ASPIRIN INDUCED ULCER MODEL

Effect of *Annona squamosa* Leaf on Aspirin induced ulcers.

Table No: 5

Groups	Ulcer Index	% Protection
GroupI - Control	18.71±0.10	--
Group II - Standard (Omeprazole)	3.79±0.07***	79.7 %
Group III – *Annona squamosa* Extract 100mg	6.47±0.08**	65.41%
Group IV – *Annona squamosa* Extract 50mg	8.74±0.08**	53.28 %

All values represent Mean ± SEM, n=6 in each group. ***p<0.001, **p<0.01, Control group is compared with standard and extract doses. Data were analyzed by one-way ANOVA followed by Dunnett's test

GRAPHICAL REPRESENTATION

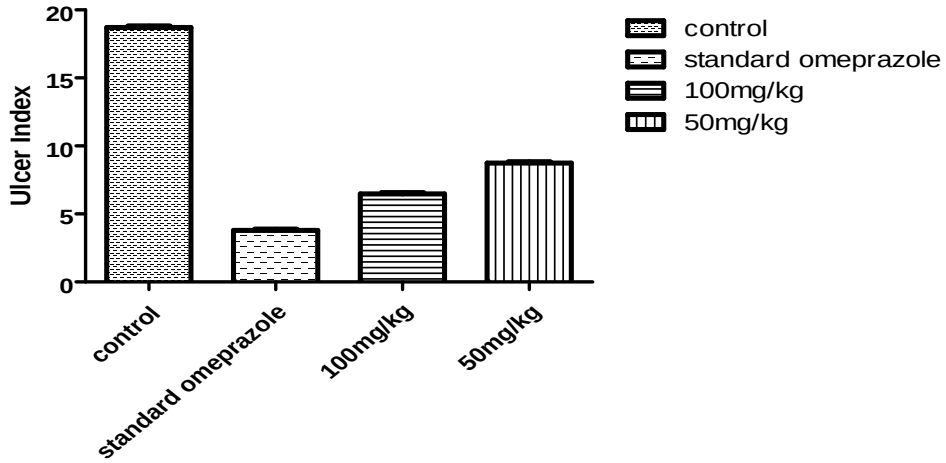


Figure: 5

Figure: 5 showing effect of extract of *Annona squamosa* on ulcer index

Effect of *Annona squamosa* on Aspirin induced ulcer

1. Ulcer Control

2. Std (Omeprazole 20mg/kg)

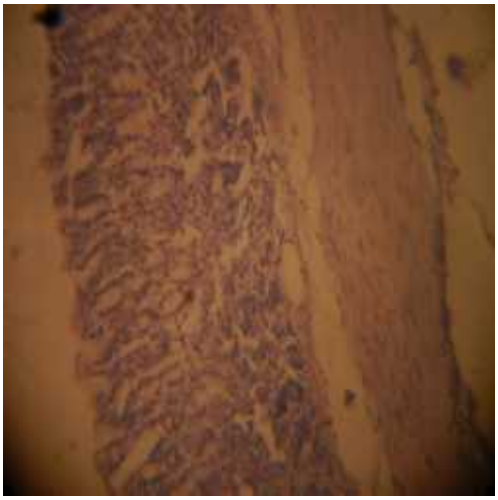
3. A.squamosa 50mg/kg

4. A.squamosa 100mg/kg.

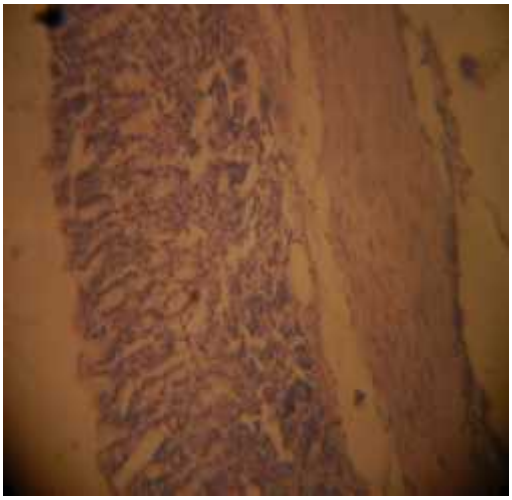
Figure: 6

(A)- Control, (B) – Standard, (C) – Extract 50mg/kg, (D) – Extract 100mg/kg.

HISTOPATHOLGY IMAGES –ASPIRIN INDUCED ULCERS

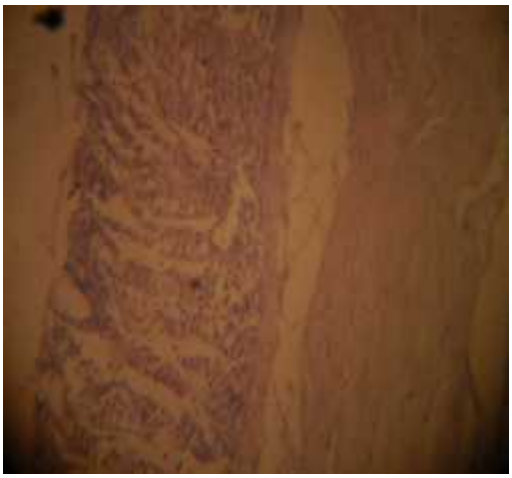


(A)



(B)

(C)



(D)

Figure: 7

(A)- Control, (B) – Standard, (C) – Extract 100mg/kg, (D) – Extract 50mg/kg.

Histopathology Examination

The images and the reports demonstrate a prominent damage, loss of mucus and chief cells as well as marked infiltration of the leucocytes to the stomach surface of the rats in group-I treated only with aspirin. In Group-II, the standard group showed no damage to the gastric mucosa. The histopathological sections of group-III, treated with ethanolic extract of *A.squamosa 100* mg/kg, shows a reduction in the ulcer focus and a hyperplastic gastric mucosa with regenerating mucosal epithelium. The section of IV- group rats shows mucosal erosion and ulceration to the stomach surface illustrating a less protection to the mucosa and the gastric epithelium.

Ulcer index (UI)

Oral administration of ethanol extract of *Annona squamosa* at doses of 50 and 100mg/kg exhibited dose dependent inhibition percentage of 53.28 and 65.41 ($p<0.001$) respectively compared to the ulcer control, proving the anti ulcer activity. The standard drug omeprazole (20mg/kg) exhibited percentage inhibition of 79.7 when compared with ulcer control. Extract treated and ulcer control group was compared with standard group.

Aspirin is a cyclooxygenase inhibitor which suppresses gastroduodenal bicarbonate secretion, reduces endogenous prostaglandin biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow in animals. It is also well known that prostaglandins synthesized in large quantities by the gastrointestinal mucosa can prevent experimentally induced ulcers by ulcerogens. **(Telesphore *et al.*, 2008)**

The effects of ethanolic extract of *Annona squamosa* on acid parameters were less significant at 50mg/kg dose. But ethanol extract of *Annona squamosa* showed significant ($p<0.001$) effect at 100mg/kg dose compared to ulcer control animals. But, in this gastric environment also able to induce ulcer, so it can be thought that the anti-secretory activity might not be the main mechanism of action of these extracts.

7.2.3.B. PYLORUS LIGATION INDUCED ULCERS

Table 6: Effect of *Annona squamosa* on pylorus ligation induced ulcers

Groups	Ulcer Index	% Protection
Group I - Control	14.64±0.10	--
GroupII - Standard (Omeprazole)	5.63±0.10***	61.54 %
Group III – *Annona squamosa* Extract 100mg	8.67±0.07**	40.77%
Group IV – *Annona squamosa* Extract 50mg	10.65±0.10**	27.25 %

All values represent Mean ± SEM, n=6 in each group. ***p<0.001, **p<0.01, Control group (Group I) is compared with standard and extract doses. Data were analyzed by one-way ANOVA followed by Dunnett's test.

GRAPHICAL REPRESENTATION

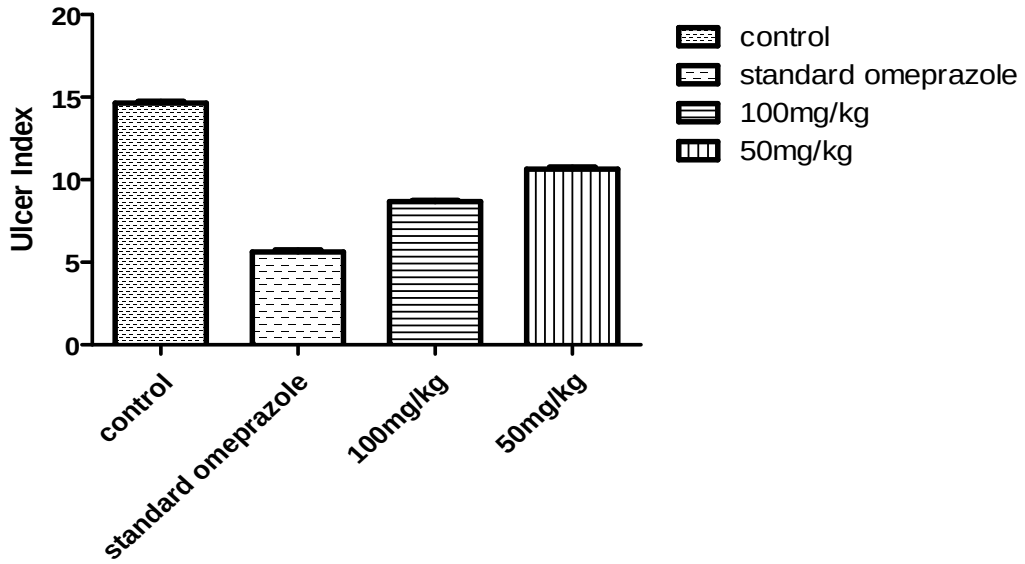
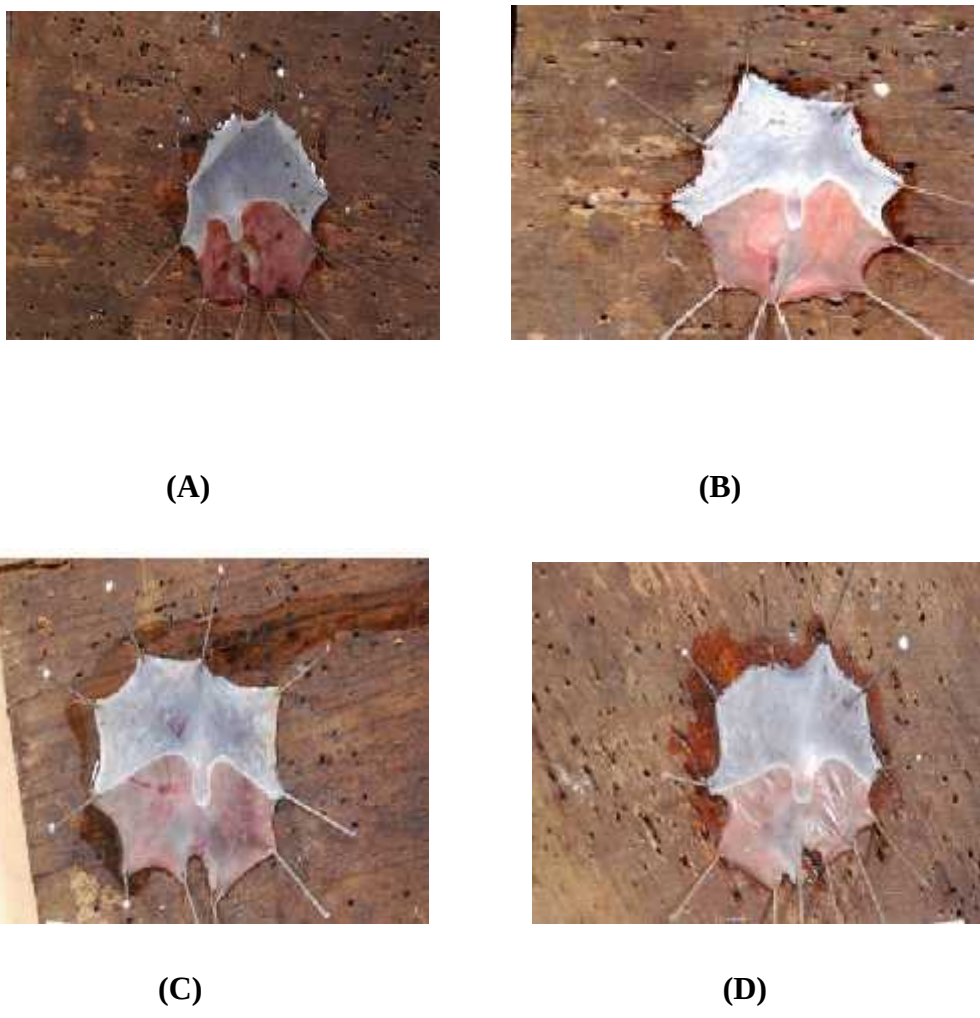


Figure: 7

Figure: 7 showing effects of extract of *Annona squamosa* on ulcer index.

Effects of extract of *Annona squamosa* on ulcer index



(A) **(B)**

(C) **(D)**

Figure: 8

(A)– Control, (B) – Standard, (C) – Extract 50mg/kg, (D) – Extract 100mg/kg.

Table No: 7 Effect of Annona squamosa on gastric secretions, free acidity and total acidity on pylorus ligation model

Group	Gastric Volume	pH	Free Acidity	Total Acidity
Control	5.56 ± 0.10	1.5 ± 0.08	65.06 ± 1.19	70.75 ± 1.21
Std(omeprazole)	1.46±0.07***	4.31±0.07***	28.95±0.96***	36.99±0.97***
Extract 100mg	2.64±0.07**	3.44±0.08***	45.71 ± 0.88**	54.04±1.11***
Extract 50mg	3.48 ± 0.01**	2.45 ± 0.09**	53.19 ± 0.54*	63.03±1.15***

All values represent Mean ± SEM, n=6 in each group. ***p<0.001, **p<0.01, *p<0.05. Control group is compared with standard and extract doses.

Data was analyzed by one-way ANOVA followed by Dunnett's test.

Oral administration of Ethanolic extract of *Annona squamosa* attenuated the gastric volume, free acidity, total acidity and ulcer index thus showing the anti secretary mechanism in table 7. The ethanol extract of *Annona squamosa* exhibited a dose dependent inhibition percentage of 27.25 and40.77 at doses of 50 & 100mg/kg dose respectively (p<0.001). The standard drug Omeprazole showed an inhibition percentage of 61.54. The extract and standard group compared with ulcer control. The results are shown in table and parameters are shown in **table 7.**

The ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, decrease in free and total acidity. It is significant to note when the pH reached 3, the ulcer score appeared less. Omeprazole was used here to study the proton pump inhibitor mechanism. Moreover the disturbance of defensive factor like mucus secretion, bicarbonate secretion and mucosal blood flow has been reported to cause ulcer. Ethanol extract of *Annona squamosa* showed a dose dependent ulcer curative ratio in pylorus ligation induced ulcers. Even though the extracts reduced

the incidence of ulcers when compared to ulcer controls, the inhibition percentage of extracts were not closer to the standard drug omeprazole,

Graph Comparing the amount of Gastric volume collected from each of the Control, Standard and the Extract (100 and 50mg/kg) groups.

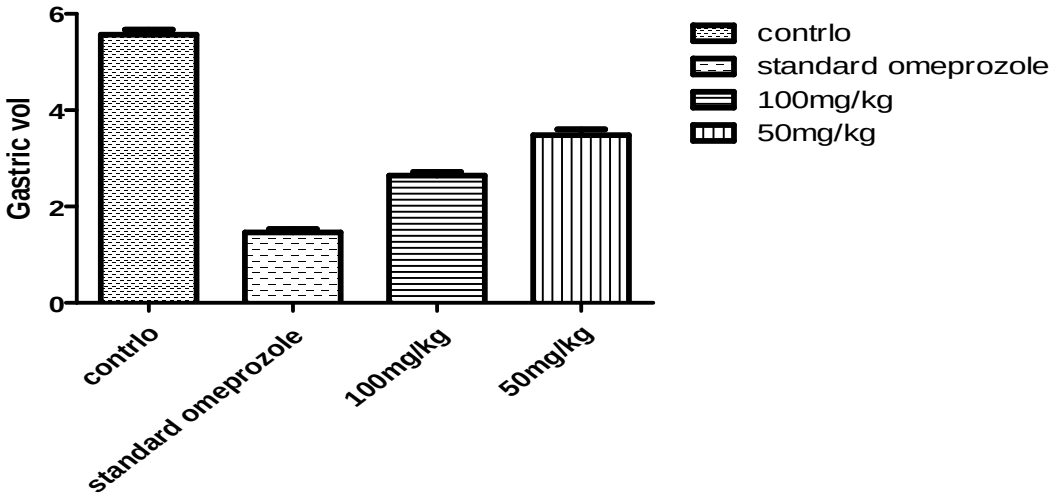


Figure: 9

Graph Comparing the pH of Gastric contents collected from each of the Control, Standard and the Extract (100 and 50mg/kg) groups

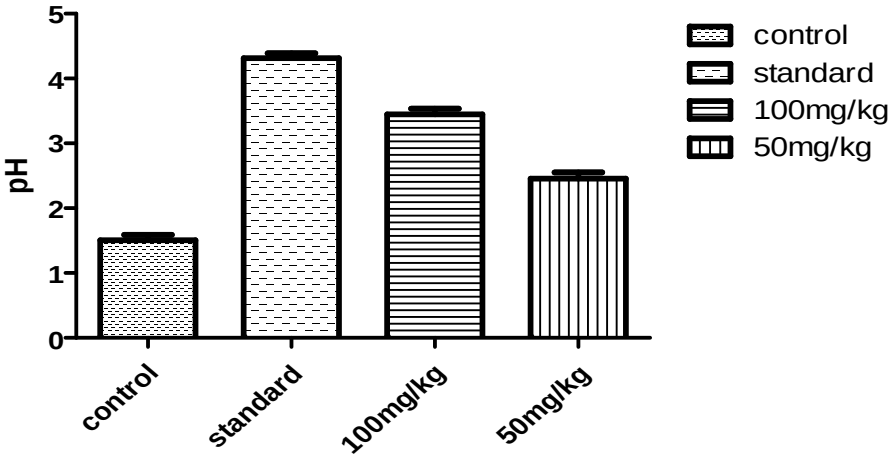


Figure: 10

Comparison of Free Acidity of the Control, Standard and the Extract (100 and 50mg/kg) groups.

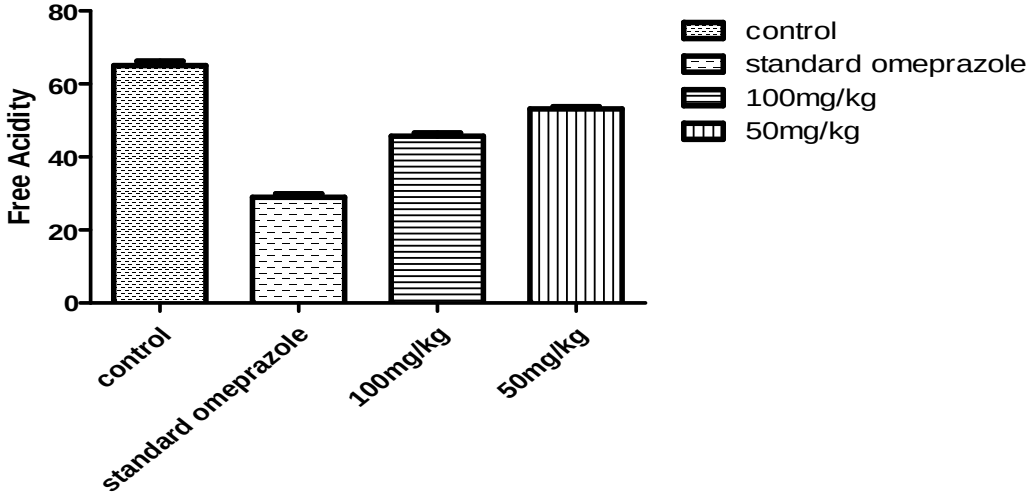


Figure: 11

Comparison of Total Acidity of the Control, Standard and the Extract (100 and 50mg/kg) groups

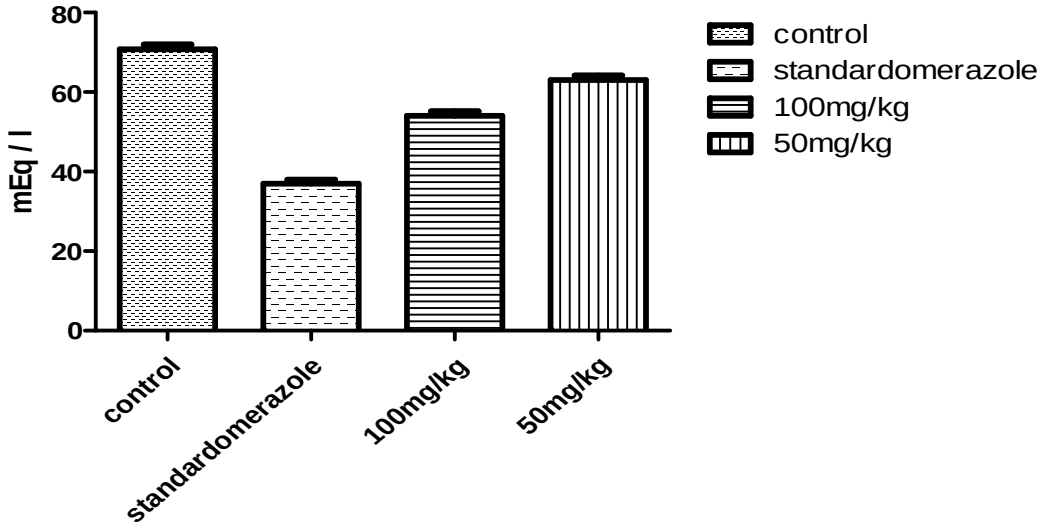
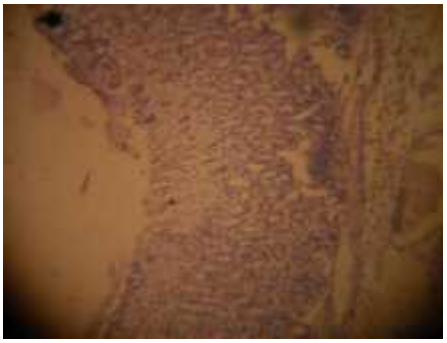
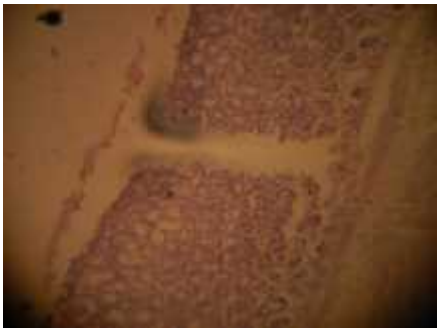


Figure: 12

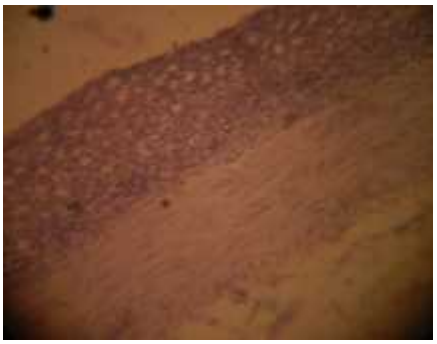
HISTOPATHOLOGY IMAGES – PYLORUS LIGATED ULCERS



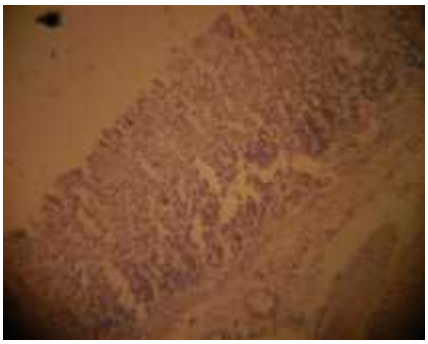
(A)



(B)



(C)



(D)

Figure: 13

(A) – Control, (B) – Standard, (C) – Extract 100mg, (D) – Extract 50mg.

Histopathology examination

The histopathological examinations of stomachs of the rats show us a better picture of the gastric lesions and the damage occured to the stomach mucosa. Significant acute ulceration of the stomach was observed in group-I which was Pylorus ligation. This is revealed from Figure (A) (control group rats). The pylorus ligation induced extensive macroscopic mucosal damage which can be known by the injury to the epithelia of the mucosa. Also, elongated hemorrhagic lesions were observed running perpendicular to the axis of the stomach. Histology of the rat stomach of the standard group pertaining to the figure (B) showed normal architecture of the gastric mucosa. The histopathological reports of the group three rats shows appearance of gastric epithelium being showed in (C). Figure (D) shows almost normal appearance of the epithelium. These observations matched well with the ulcer index parameter.

7.2.2. C ANALGESIC SCREENING

ACETIC ACID INDUCED WRITHING METHOD

Effect of *Annona squamosa* extract on acetic acid induced writhing in mice

Table No: 8

S. No	Groups	Number of writhing's	Percentage decrease in activity
1	**Control (acetic acid)**	55.00±3.32	-
2	***A. squamosa* extract 50mg/kg**	14.55±1.32*	73.5%
3	***A.squamosa* extract 100mg/kg**	7.98±0.51**	85.4%
4	**Standard(pentazocin)20mg/kg**	6.48±0.54***	88.2%

All the values are expressed as mean ± S.E.M, (n=4) animals in each group***p<0.001, **p<0.01and *p<0.05. Extract treated and standard groups were compared with control.

Data were analyzed by one-way ANOVA followed by Dennett's test.

GRAPHICAL REPRESENTATION

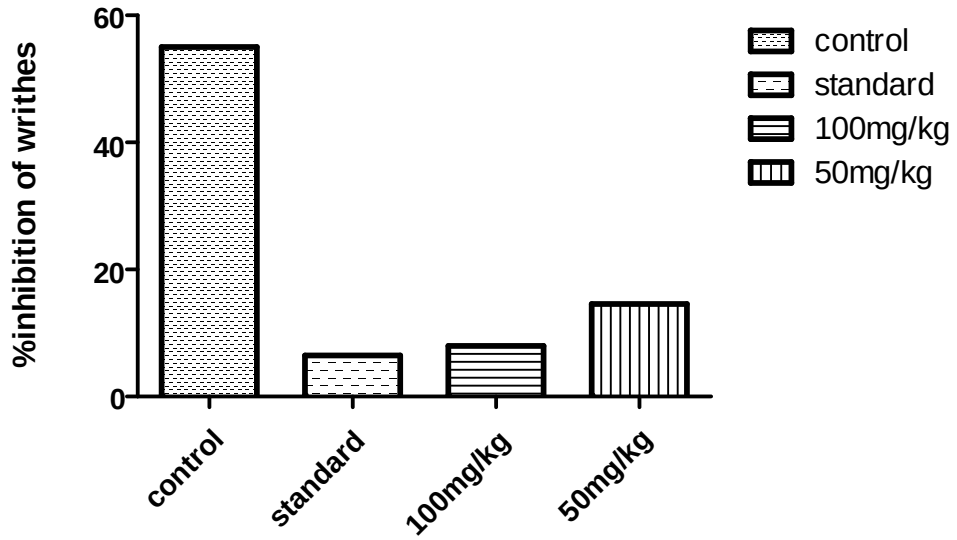


Figure: 14

Effect of ethanolic extract of *Annona squamosa* on writhes induced by acetic acid in mice shown in **Table 8.**

The result of effect of *Annona squamosa* on writhing method in mice is given in **table 8**. The ethanolic extract produced significant analgesic activity in dose dependent manner and a significant effect was observed at a dose of 100 mg/kg. The result of the data reflects the intensity of analgesic activity at peripheral area, which is compared with standard drug (pentazocine).

Drugs, which are centrally both peripheral and central, such as petazocine, only inhibit the late phase. A number of alkaloids have been reported to produce analgesic activity.

The pharmacological studies of extract showed that, extract possessed analgesic activity to varying extent. It showed that alkaloids present in these extract may be responsible for the pharmacological action. The extract was tested at two different doses (50 and 100mg/kg) to know if they were dose dependent.

First the control group of animal were tested with the acetic acid, and then observed the number of writhes produced by the animal. The extract exhibited an percentage inhibition of 73.5 and 85.49 at doses of 50 and 100 mg/kg respectively. These shows that ethanolic extract of *Annona squamosa* shows high significantly decrease in the writhing response induced by acetic acid when compared with control group.

.

DISCUSSION

Most of the studies demonstrate the importance of natural products in drug discovery. In these study antiulcer and analgesic activities of ethanolic extract of *Annona squamosa* has been studied. The antiulcer study was evaluated using aspirin and pylorus ligation models whereas analgesic activity was evaluated by acetic acid induced writhing model.

Most of the studies demonstrate the importance of natural products in drug discovery. The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs. The acute oral toxicity study result showed that the plant leaf is safe.

Peptic ulcer describes a condition in which there is a discontinuity in the entire thickness of the gastric and duodenal mucosa that persists as a result of acid and pepsin in gastric juice. Peptic ulcer disease (PUD) is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy. It includes number of drugs such as proton pump inhibitors and H2 receptor antagonists are available for the treatment of peptic ulcer, Peptic ulcer occurs due to an imbalance between aggressive (acid, pepsin) and defensive (gastric mucosal barrier) factors of gastric mucosa.

Aspirin induced model shows significant percentage inhibition when compared with standard. As aspirin is COX inhibitors suppress gastro duodenal bicarbonates secretion and endogenous prostaglandin biosynthesis, disrupts mucosal barrier. The ulcer index parameter was used for the evaluation of ulcer activity. Moreover the disturbance of defensive factor like mucus secretion, bicarbonate secretion and mucosal blood flow has been reported to cause ulcer.

Pylorus ligation model is usually employed to observe the potential of anti ulcer drugs for their anti-secretory activity by checking the gastric volume and its effect on gastric pH, total acidity and free acidity. Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier and also because of an increase in acid-pepsin accumulation due to pylorus obstruction and subsequent mucosal digestion.

. The pharmacological studies of extract showed that, extract possessed analgesic activity to varying extent. It showed that alkaloids present in these extract may be responsible for the pharmacological action. The analgesic activity may be attributed by the presence of various phytoconsitiuents such as alkaloids, tannins, carbohydrates, flavonoids. Antinociceptive analgesic activity of *Annona squamosa* was evaluated using chemical model of nociception inice. Acetic acid induced writhing test is used for detecting both central and peripheral analgesics as these method is very sensitive and able to detect antinociceptive effect of compounds at dose levels. The intraperitoneal administration of acetic acid produces abdominal writhing response due to sensitization of chemo sensitive nociceptors by PGs. It is therefore possible that the extract exerts their analgesic effect probably by inhibiting the synthesis or action of PGs.

8. SUMMARY AND CONCLUSION

Summary

In the present work medicinally important active constituents of dried leaves of *Annona squamosa* were studied with special emphasis on biological activities.

Pharmacognostical, Preliminary phytochemical and Pharmacological activities were studied and reported.

Histopathological observation for antiulcer activity was done on abdominal section. Antiulcer and analgesic activities were studied.

Conclusion

It was found that antiulcer activity exhibited was due to mucosal defensive factor. Hence it can be used for management of peptic ulcer. Analgesic activity was exhibited to the level of clinical importance. It was found that the activity was due to the presence of flavonoids.

Chemical substances derived from plant have got a very long history in treatment of human diseases. Nearly 50% of new chemical entities introduced during the past two decades were from natural products.

Further research is required to isolate the active phytoconstituents present in the extract and experimentation on the healing action of drug on chronic ulcer as well as on the possible side effects. The investigation on mode of action may pave way for establishment of new anti-ulcer therapy regimen.

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